

IS-MPMI *Reporter*

International Society for Molecular Plant-Microbe Interactions
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Update on Progress of Your Journal: Molecular Plant-Microbe Interactions

Stanton B. Gelvin, Editor-in-Chief

As many of you know, Molecular Plant-Microbe Interactions (MPMI) is your official Society journal. I have been privileged during the past three years to be the Editor-in-Chief. During this time, MPMI has seen a lot of growth and gone through a number of changes. I am extremely pleased with the progress the journal has made and its reputation in the scientific community. This progress could not have occurred without the help of a large number of individuals, including (but not limited to) the American Phytopathological Society (APS) Press staff (Paul Hintz, Pam Johnson, Steve Nelson, Ina Pfefer, Jean Rice, and Miles Wimer) and the past and current Senior Editors (David Baulcombe, Jan Leach, Peter Palukaitis, John Ryals, Gary Stacey, and Jon Walton).

As I started collecting data to write my final report, I looked over some past reports and, particularly, a memo that I wrote in the autumn of 1994 before taking over as Editor-in-Chief. This memo was entitled "Goals for MPMI Editorial Board", and included my "wish list" of things to accomplish during my tenure as EIC. As I read again these goals, I was both very happy with and a bit astounded by how much we actually had done. The items included:

1. Increase the number of issues of MPMI from 6 per year (the number published in 1994 and 1995) to 9 per year, then 12 per year. Because of a large increase in the number of submissions of high quality manuscripts, MPMI started publishing 9 issues per year in 1996, and has continued at this rate in 1997. In 1998, MPMI will publish 12 issues per year. The monthly publication of MPMI was my main goal as EIC, and I am especially pleased that we have accomplished this. Special thanks, of course, go to all the scientists who have submitted their high-quality work to the journal.
2. Decrease the time from manuscript acceptance to publication. Because of numerous changes at the Headquarters office, we have decreased manuscript processing time (after acceptance) from greater than 130 days in 1994 and 98 days in 1996 to a 1997 average of 83 days. In addition, changes in manuscript routing procedures have resulted in decreased time for review. Probably the major contributing factor in

the time from submission to acceptance is the time authors take to make revisions in manuscripts.

3. Revitalize the associate editorial board. In 1995, the Senior Editors met at the headquarters in St. Paul, Minnesota and participated in the first MPMI Senior Editor "training session". At this session, we reorganized the Associate Editor board. Associate Editors were placed on 3-year rotating appointments, with approximately 1/3 of the board rotating off each year. We additionally reconstituted the board so that its members more closely reflected the spectrum of manuscripts that were being submitted and published. Special attention was given to assure that the board reflected the international nature of the journal, and the large number of female scientists working in our field.
4. Increase emphasis on Plant Response, Biological Control, and Nematode-Plant Interactions. There has been an increase in the number of papers published particularly in the areas of Plant Response and Nematode-Plant Interactions. To accommodate this broadening of research emphasis, we have created a Senior Editor position for Plant Response and 4-5 Associate Editor positions in this area. We have appointed one Associate Editor each in the areas of Biological Control and Nematode-Plant Interactions. We look forward to increased submissions in each of these three research areas in the future.
5. Increase the number of submissions of high quality manuscripts. We have made exciting progress in this area. In 1989, 82 manuscripts were submitted. The number has climbed steadily to 209 in 1996 and 106 for the first six months of 1997. There has been a 30% increase in manuscripts submitted during the past 3 years. This has allowed us to increase the number of issues per year from 6 to 12.
6. Remain competitive with other journals in the field. MPMI is rated number 3 among the primary literature plant journals in the number of citations per article, only behind *The Plant Cell* and *The Plant Journal*. These latter two journals are more "general" plant molecular and cell biology journals; MPMI is a more "specialized" journal. In order to compete more effectively with these (and other) journals, we have lowered our charges for publication of color figures to rates comparable to or less than competitor journals. In 1996, 31 color figures were published in 22 different articles. In 1997 (the first 5 issues) 38 color figures were published in 25 different articles. In addition, more rapid review and publication schedules (MPMI has frequently come out AHEAD of schedule) has made MPMI an attractive journal in which to publish. In 1998, MPMI will be published "on line" in addition to "hard copy". We hope that this will additionally increase the attractiveness of the journal. Finally, the publication of an increasing number of articles in the area of Plant Response to Microbes makes MPMI a leading journal in which to publish in this area of research.

In January, 1998, Dr. Jan Leach will become Editor-in-Chief of MPMI. I would like to take this opportunity to wish her all the best in her new duties, and to encourage all of you to continue to support your journal.

Research Notes

RECA-AC for Genome Studies of Plants and Microbes

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Genome cleavage methods that are based on the use of site-specific DNA binding agents to protect restriction sites from methylation were pioneered in the laboratory of Dr. Waclaw Szybalski at the University of Wisconsin. These methods serve to create a uniquely cleavable "weak spot" in the genome and for this reason the term "Achilles' Cleavage" (AC) was invoked (1). These techniques have proven

extremely useful for the physical mapping of several regions of the Magnaporthe grisea genome (2) and were also central to the successful cloning of an M. grisea avirulence gene (3). RecA-Assisted Achilles' Cleavage (RecA-AC) potentially enables specific cleavage of any restriction site for which a corresponding methylase is available. For this reason, it is broadly applicable to a wide variety of organisms and should greatly expedite physical mapping studies and positional cloning efforts. Potential applications and technical aspects are discussed in this review.

How RecA-AC Works

The *in vivo* function of the *Escherichia coli* RecA protein is to promote homologous recombination of DNA. Consequently, it performs a variety of functions related to the mediation of associations between DNA molecules. The function utilized in RecA-AC is the assimilation of single stranded DNA into homologous regions of duplex DNA to create a transient triplex structure. In RecA-AC, this reaction is performed *in vitro* as outlined in Figure 1A. The starting material for all these studies is intact chromosomal DNA embedded in agarose microbeads. An oligonucleotide that is complimentary to sequences surrounding a chosen cleavage site is mixed with RecA protein to form a nucleoprotein filament. This filament is diffused into the microbeads to initiate triplex formation with the genomic DNA. A non-hydrolyzable analog of ATP, ATP S, is added to the reaction to stabilize the triplex and a DNA methylase is then added to modify restriction sites not protected by the RecA:DNA complex. Finally, deproteinization of the complex reconstitutes the parental strands enabling subsequent restriction of the DNA. Figure 1B illustrates the typical results of an AC reaction.

Why use RecA-AC?

RecA-AC offers several advantages over other long-range physical mapping techniques. Rare-cutting restriction enzymes are often used to cut DNA into fragments whose sizes range from hundreds of kilobases up to a megabase or so. The fragments are then resolved by CHEF or FIG electrophoresis and Southern hybridization analyses are performed to determine if different probes bind to the same fragments. This approach entails a lot of effort to find enzymes that digest rarely enough that different probes exist on the same fragment but often enough that a maximal distance between them can be approximated. Ultimately, using this approach one can only determine the maximum distance between two sequences. Furthermore, it is possible that probes may hybridize to similarly-sized but non-identical restriction fragments.

Clearly, it is more desirable to cleave the genome uniquely (or at few loci) within known sequences. Being able to effect such precise control over cutting locations necessitates that endogenous cleavage sites do not exist in the genome.

Two approaches have been used to fulfill these requirements: I) engineering of high-specificity cleavage sites into the genome; and II) enzymatic modification of DNA outside the chosen cut site. Intron-encoded nucleases typically have large recognition sites which may span 20 to 30 base pairs. The frequency with which these sequences would occur randomly is exceedingly small (1 in 420 to 1 in 430 bases). Similarly, lambda terminase cleaves a large target. Introduction of such sequences into the genome therefore tends to create unique cleavage sites. However, mapping of specific loci requires that the recognition sequence be introduced into the target region by trans-formation-mediated homologous recombination. While this approach has been used to great effect for mapping toxin loci in the phytopathogenic fungus *Cochliobolus carbonum* (7), other organisms may be less obliging in their propensity to effect homologous integration of transforming DNA. For this reason, this

approach is not considered generally applicable. A second approach to achieving specificity is to protect the chosen target site and then render the rest of the genome uncleavable. Several DNA-binding proteins have been used to protect restriction sites (8,9). However, this strategy also suffers from the problem that recognition sites have to be introduced into the genome under study. Enter RecA protein. RecA protein provides an ideal system for site-specific protection of DNA because its specificity for DNA is determined not by the protein itself but by the affinity of its single-stranded DNA substrate for regions of complementarity. Thus the oligonucleotide seeks out its complement in the genomic DNA and when it succeeds, RecA protein assimilates the oligonucleotide into the duplex strand to form a triplex and acts to stabilize the complex until experimentally inactivated. Clearly, the degree of specificity can be adjusted by modifying the length of oligonucleotide. While the fidelity of these associations are highly dependent on factors such as oligonucleotide/RecA protein ratios, magnesium ion concentrations and maintaining reducing conditions in the reaction mixture; under optimal conditions RecA experiments may be performed with high reproducibility. Regrettably the efficiency of protection from methylation is not 100%. It follows that some of the DNA will not be cleaved when the final restriction digestion is performed. However, this is rarely of any concern as the yield of product is typically plentiful enough to be observed on an agarose gel and to be efficiently subcloned into a plasmid vector.

Experimental Details

Specific experimental conditions for RecA cleavage have been outlined previously and may be found on the Fungal Genetics Stock Center Web site at: <http://www.kumc.edu/research/fgsc/methods/farman.html>. Some additional hints are provided below to increase the likelihood of success.

The original protocol calls for intact chromosomal DNA embedded in agarose microbeads. These are relatively easy to make but if they are not available or prove difficult to obtain, it is possible to use agarose plugs or tubes if these are first sliced with a coverslip. When equilibrating the beads with buffer, care should be not to lose beads at each exchange. This may ultimately result in having so few beads that the DNA does not show up on the gel.

Essentially, it is possible to achieve absolute specificity for cleavage at chosen site(s) and because the sequence of the oligonucleotide directs the strand association, cleavage may be targeted to any restriction site for which a corresponding methylase is available. In reality, some restriction sites are more favorable targets than others because certain commercially available methylases are difficult to obtain nuclease-free (4). Clearly nuclease contamination would seriously compromise long range mapping/cloning strategies. For this reason, most researchers utilizing RecA-AC have chosen to target EcoRI sites in their studies (2,5,6).

In my hands, an oligonucleotide: RecA protein ratio of 0.165 μ g:6.25g has worked well at all loci studied. However, depending on its sequence content or the source of RecA protein, it may be necessary for others to titrate the oligonucleotide.

To become familiar with the techniques and to gain confidence in its effectiveness, I encourage first time users to attempt the procedure first on a plasmid containing the target locus. This is a useful positive control and is an easier substrate to handle when titrating oligonucleotide against RecA protein.

Care should be taken to ensure that the magnesium acetate and dithiothreitol concentrations are adjusted as indicated throughout the protocol. It is easy to overlook these subtleties but they are critical to the success of these experiments. The dithiothreitol solution should be freshly prepared.

When stepping up to genomic DNA substrates, arriving at appropriate electrophoresis conditions to resolve cleavage products from intact chromosomal DNA may require some trial and error. It is advisable to test first the efficacy of the cleavage by digesting the genomic DNAs (controls + experimental samples) with a second enzyme that is insensitive to methylation. Southern blotting should reveal whether the (second enzyme) fragment containing the target site has been efficiently cleaved. This is favorable to trying several conditions to resolve cleavage products from intact DNA, only to find that the reaction did not work in the first place!

Demonstrated uses of RecA-AC Mapping distances between RFLP markers.

In the laboratory of Dr. Sally Leong, RecA-AC was applied to eight loci in the genome of the filamentous fungus *Magnaporthe grisea*. Six of these cleavages were directed to sites surrounding the AVR1-CO39 avirulence gene locus. By adding two oligonucleotides to each reaction we were able to cut the genome simultaneously within separate markers enabling us to measure the distance between them. In this manner we assembled a high resolution physical map of the avirulence gene locus (3).

Monitoring of a chromosome walk.

The correct orientation of a chromosome walk to the *M. grisea* avirulence gene locus was rapidly established by hybridizing cosmid end clones to Southern blots of Achilles' cleaved DNA. During the walk, we also encountered several potential obstacles such as chimeric cosmid clones, middle repetitive DNA and rearranged clones. These led to an occasional hop on to another chromosome. However, as newly identified endclones were continually hybridized to AC blots, these diversions were immediately detected and the walk was kept "on track."

Targeted cloning of a specific chromosome segment.

When the chromosome walk was curtailed due to: i) absence of overlapping clones in each of several libraries and ii) a region of highly repetitive DNA; AC was used to release the chromosomal segment between these problem areas. This piece of DNA was resolved by electrophoresis, purified and then subcloned into a plasmid vector. The resulting clones yielded new entry points enabling the walk to be continued throughout the target region. This ultimately led to the cloning of AVR1-CO39 (3).

Partial digestion of genomic DNA.

We investigated how RecA-AC would work if the target sequence was present in multiple copies in the genome. An oligonucleotide was synthesized that was complementary to a region overlapping an *EcoRI* site in the MAGGY retrotransposon (10). RecA-AC of genomic DNA of strain 2539 which possesses more than 40 copies of MAGGY yielded a ladder of cleavage products after electrophoresis. Hybridization of selected probes to Southern blots of these gels revealed interelement and element to telomere distances. Furthermore, the partial digestion created by incomplete protection, in some cases enabled the adjacent interelement distance to be determined. Thus, AC at repetitive DNA loci provides another useful physical mapping tool.

Separation of chromosomal segments for further analysis.

RecA-AC products that had been resolved by CHEF electrophoresis were excised from the agarose and

digested *in situ*: with conventional 6-base cutting restriction enzymes. The agarose plugs were then inserted into the wells of a second gel and electrophoresed adjacent to a well of total genomic DNA digested with the same enzyme. Blots of these gels were then hybridized with probes from repetitive DNAs such as telomere repeats or transposons. In this manner, we were able to determine which telomeric restriction fragment resided on a selected cleavage product. Similarly, it was determined that an unmapped portion of *M. grisea* chromosome 3 (2) contained at least 2 copies of the *GRASSHOPPER* retrotransposon (11).

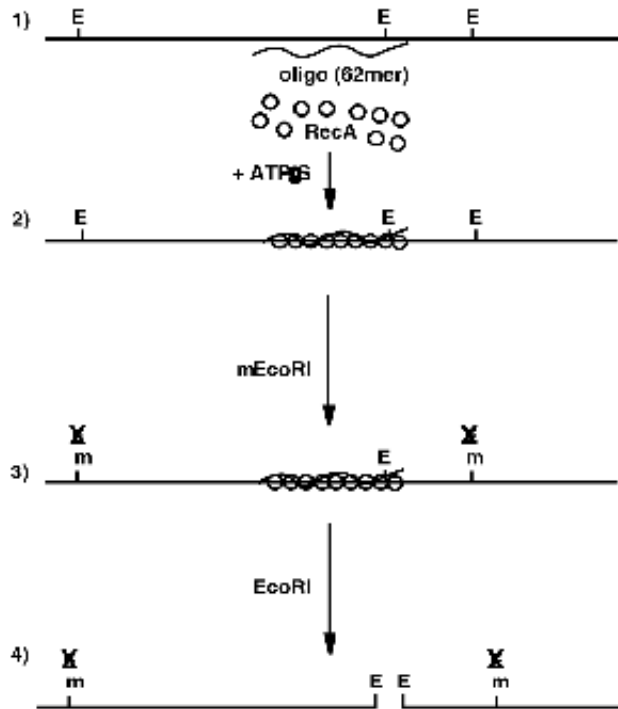
Targeted cloning of whole Achilles' cleavage products.

Experiments are in progress in the laboratories of Drs. Koob and Szybalski to develop techniques enabling the ligation of intact fragments released by RecA-AC. YAC and BAC vectors with long single-stranded tails that are complementary to the ends of the cleavage products can be used to provide targeted cloning of cleavage products whose ends have been recessed by lambda exonuclease (4, 12).

1. Science (1988) 241: 1084-1086
2. Genetics (1995) 140:479
3. Farman and Leong, submitted Genetics
4. M. Koob (koobx001@gold.tc.umn.edu)
5. Koob et al (1992) Nucl. Acids Res. 20: 5831-5836
6. Science (1991) 254: 1494-1497
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8. Gene (1992) 110: 1-7
9. Gene (1990) 90: 1-7
10. Mol. Gen. Genet. (1996) 251: 665-674
11. MPMI (1993) 6: 114-126
12. W. Szybalski balsk@macc.wisc.edu)

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A)



B)

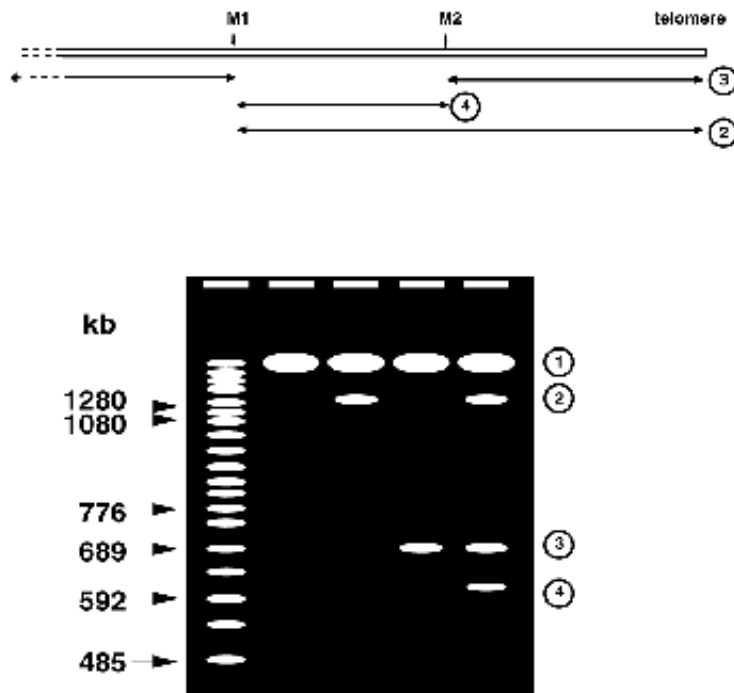


Fig. 1. Principle of RecA-AC. A) An oligonucleotide is synthesized that is complementary to DNA overlapping an *EcoRI* site. Microbead-embedded genomic DNA is incubated with RecA protein, the oligonucleotide and a non-hydrolyzable analog of ATP (1), causing a triplex to form over the restriction site (2). The DNA + complex

is treated with *EcoRI* methylase (3) and then the triplex is denatured. The DNA is digested with *EcoRI* (4) and then analyzed by CHEF electrophoresis (Fig. 1B). B) Results of a typical RecA-AC double cleavage reaction surrounding the *M. grisea* AVRI-CO39 locus. Upper panel shows physical map of the region under study with the targeted cleavage sites indicated with arrows. Lower panel shows a schematic of a typical ethidium bromide-stained gel. The first lane represents a lambda DNA ladder marker. Lane 2 contains a RecA cleavage reaction to which no oligonucleotide or RecA protein was added. The entire genome becomes methylated and is uncut by subsequent *EcoRI* digestion. Undigested chromosomes and very large cleavage products tend to migrate together under most CHEF conditions (band 1). Lane 3: RecA + oligo "A" added. Methylation inactivates all genomic *EcoRI* sites except the one covered by oligo "A." Subsequent *EcoRI* digestion releases a 1.29 Mb fragment (band 2). Lane 4: RecA protein + oligo "B." Protection and cleavage of site B releases a 680 kb fragment (band 3). Lane 5: RecA + oligo "A" + oligo "B." Simultaneous protection of sites A and B yields fragments of 610 kb (band 4) and 680 kb (3). A third cleavage product of 1.29 Mb is visible in Lane 5 due to incomplete protection by oligo B (incomplete protection by A yields a large fragment that comigrates with the undigested chromosomal DNA and other large cleavage products)

Employment Listings

You can access current employment listings or advertise an available position through the IS-MPMI job placement service. This service is free of charge and can be accessed through the IS-MPMI homepage located at: <http://www.scisoc.org/ismpmi>

If you would like more information about the IS-MPMI job placement service or need to receive your member password to access the listing via the Internet, contact: Maureen Mullin, IS-MPMI Job Placement Service Manager, IS-MPMI Headquarters, 3340 Pilot Knob Road, St. Paul, MN 55121-2097; Phone: +1-(651) 454-7250 Fax: +1-(651) 454-0766
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Membership Directory

Although this is the member directory issue, membership is required to access the directory.

If you would like more information about IS-MPMI membership or need to receive your member password to access the on-line directory, contact: Denise Kessler, IS-MPMI Member Services, IS-MPMI Headquarters, 3340 Pilot Knob Road, St. Paul, MN 55121-2097; Phone: +1-(651) 454-7250 Fax: +1-(651) 454-0766
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IS-MPMI Publication

Biology of Plant-Microbe Interactions

Edited by Gary Stacey, Beth Mullin, and Peter M. Gresshoff

This collection of studies is an overview of recent advances in the intriguing field of molecular plant-microbe interactions research. Each chapter was contributed by participants in the Eighth International Congress on Molecular Plant-Microbe Interactions which was held in Knoxville, Tennessee, USA in July 1996.

This book should prove useful for researchers, scholars, and students interested in the most recent advances in the study of plant-microbe interactions. The chapters are written by world authorities on information that has been gathered during the last two years using modern molecular methods.

These studies draw on a wide variety of scientific disciplines, including biology, chemistry, biochemistry, and cell biology. Topics covered include molecular studies of plant symbioses, *Agrobacterium*, bacterial and fungal pathogens, microbial signals and signal transduction pathways, biocontrol, and applications to biotechnology. A section focusing on emerging areas of research on plant-microbe interactions is also included. 1997; 6" x 9"; hardbound; 608 pages, 16 black and white photographs, 88 figures, and 22 tables; ISBN: 0-9654625-0-1

IS-MPMI Member: \$140 Non-member Price: \$155 Student Member Price: \$40 IS-MPMI Non-member Student Price: \$65

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A Note From The Editors

We invite you to submit your material for publication in the *IS-MPMI Reporter*. We also welcome your suggestions and ideas for future articles.

The IS-MPMI Reporter 1997 schedule is:

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