The *Mla* (Powdery Mildew) Resistance Cluster Is Associated With Three NBS-LRR Gene Families and Suppressed Recombination Within a 240-kb DNA Interval on Chromosome 5S (1HS) of Barley

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ABSTRACT

Powdery mildew of barley, caused by *Erysiphe graminis* f. sp. *hordei*, is a model system for investigating the mechanism of gene-for-gene interaction between large-genome cereals and obligate-fungal pathogens. A large number of loci that confer resistance to this disease are located on the short arm of chromosome 5(1H). The *Mla* resistance-gene cluster is positioned near the telomeric end of this chromosome arm. AFLP-, RAPD-, and RFLP-derived markers were used to saturate the *Mla* region in a high-resolution recombinant population segregating for the (*Mla6* + *Mla14*) and (*Mla13* + *Ml-Ru3*) resistance specificities. These tightly linked genetic markers were used to identify and develop a physical contig of YAC and BAC clones spanning the *Mla* cluster. Three distinct NBS-LRR resistance-gene homologue (*RGH*) families were revealed via computational analysis of low-pass and BAC-end sequence data derived from *Mla*-spanning clones. Genetic and physical mapping delimited the *Mla*-associated, NBS-LRR gene families to a 240-kb interval. Recombination within the *RGH* families was at least 10-fold less frequent than between markers directly adjacent to the *Mla* cluster.

GENES in plants that confer resistance to fungal pathogens frequently display characteristic gene-for-gene specificity as originally described by Flor (1956). In nature, there are many resistance (*R*) genes in the host, each with unique specificities to particular pathogen isolates. These *R* genes are often tightly linked or represented by many alleles. The specificities among host-resistance determinants and their corresponding pathogen isolates have been useful for the genetic analyses of several resistance-gene clusters (Shepherd and Mayo 1972; Paran *et al.* 1991; Dickinson *et al.* 1993; Jones *et al.* 1993; Sudupak *et al.* 1993; Kesseli *et al.* 1994; Lawrence *et al.* 1995; Richter *et al.* 1995; Hu *et al.* 1996; reviewed by Anderson *et al.* 1997).

A large number of *Ml* specificities, which confer resistance to the powdery mildew fungus, *Erysiphe graminis* f. sp. *hordei*, have been identified in barley, *Hordeum vulgare* L. These variants are distributed among 11 groups: *Mlat*, *Mla*, *Mlk*, *Mlnn*, *Mlra*, *MlGa*, and *Mlp* on chromosome 5 (1H; reviewed by Jørgensen 1994), *Mlg* and *mlo* on chromosome 4 (4H; Görg *et al.* 1993; Büschges *et al.* 1997), *MlLa* on chromosome 2 (2H; Giese *et al.* 1993), and *Mlh* on chromosome 6 (6H; Jørgensen 1994). Thirty-two specificities at the *Mla* locus have been differentiated by their specific reaction to unique isolates of *E. graminis* (Giese 1981; Giese *et al.* 1981; Wise and Ellingboe 1983, 1985; Jahoor and Fischbeck 1993; reviewed by Jørgensen 1994; Kintzios *et al.* 1995). Hence, due to its highly variable nature, the *Mla*-resistance cluster is an excellent model for the investigation of specific recognition in gene-for-gene interactions among small grains and obligate fungal pathogens (Keen 1990; Thompson and Burdon 1992; Crute and Pink 1996). In our earlier studies, we developed a high-resolution recombinant population (selected from 3600 gametes) that makes possible the simultaneous analysis of a number of specificities of the *Mla* cluster (Mahadevappa *et al.* 1994). Of the 32 *Mla* specificities, the *Mla6*, *Mla14*, *Mla13*, and *Ml-Ru3* variants present in this recombinant population are all flanked by the *Xbcd249.1* and *Xmwg036* RFLP loci (DeScenzo *et al.* 1994; DeScenzo and Wise 1996).

In preparation for positional-cloning of the *Mla* locus, we used random amplified polymorphic DNA (RAPD; Williams *et al.* 1990), amplified fragment length polymorphism (AFLP; Vos *et al.* 1995), restriction fragment length polymorphism (RFLP; Botstein *et al.* 1980), and sequence-tagged site (STS) methods to saturate the *Mla* region with molecular markers. We used these markers to identify yeast artificial chromosomes (YACs) from the cultivar Franka, and bacterial artificial chromosomes

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(BACs) from the cultivar Morex, that are tightly linked to and spanning the *Mla* cluster. At least 11 copies of nucleotide-binding site/leucine-rich repeat (NBS-LRR) resistance-gene homologues (*RGH*s) were identified from the *Mla*-spanning, Morex BAC contig. The 11 *RGH*s are present in three distinct families and are dispersed throughout the 240-kb, *Mla*-spanning region.

MATERIALS AND METHODS

Overview of the high-resolution mapping population: The barley lines used to set up the original cross for the mapping population are nearly isogenic, differing by one or more unique *Mla* specificities in the introgressed region (Moseman 1972). Each of the lines was characterized quantitatively for its respective infection kinetics and resistance specificity (Wise and Ellingboe 1983). Crosses were constructed between the Franger- [cereal introduction (C.I.) 16151] and Rupee-derived (C.I. 16155) isogenic lines. C.I. 16151 contains the *Mla6* and *Mla14* specificities for resistance to *E. graminis*, whereas C.I. 16155 contains *Mla13* and *Ml-Ru3* (Jørgensen 1994). The flanking endosperm storage-protein-encoding genes, *Hor1* and *Hor2*, were used to select for genetic recombinants in the *Mla* region. These polypeptides are distinctly polymorphic between the lines containing different *Mla* alleles, and recombinant phenotypes can be readily visualized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of endosperm tip extracts (Doll and Andersen 1981). A total of 1800 F_2 seeds (representing 3600 F_1 gametes) were screened by this method. The final population presently consists of 286 individual F_4 homozygous lines, each representing an independent recombination event between the *Hor1* and *Hor2* loci, which are 8.1 cM apart and bracket the *Mla* cluster (DeScenzo *et al.* 1994; Mahadevappa *et al.* 1994).

Powdery mildew resistance screening: Infection types (IT) were scored as described in Mahadevappa *et al.* (1994). The infection types 0, 1, or 2 are considered resistant reactions while the infection types 3 or 4 are considered susceptible (Wise and Ellingboe 1983). The Franger- (C.I. 16151), Rupee- (C.I. 16155), Kwan (C.I. 16143, containing *Mlk*)-derived lines, in addition to Manchuria (C.I. 2330), were used as controls (Moseman 1972). Families that segregated with any isolate were retested with at least 16 individuals per line. Sixteen individuals were used to ensure 99% probability of observing at least one homozygous recessive individual (Mather 1951).

Bulk design: A 3-cM window bracketing the *Mla* cluster was defined via the recombination breakpoints in our high-resolution, recombinant population (DeScenzo *et al.* 1994). We used bulk segregant, RAPD, and AFLP analyses (Giovannoni *et al.* 1991; Michelmore *et al.* 1991; Churchill *et al.* 1993; Vos *et al.* 1995) to compare pools of 14 (for RAPD) or 16 (for AFLP) DNAs that were homogeneous within the window for either the *Mla6* and *Mla14* or the *Mla13* and *Ml-Ru3* resistance specificities.

RAPD and STS analysis: RAPD analysis was carried out using 10-base oligonucleotide primers synthesized from both Operon Technologies (Alameda, CA) and Oligonucleotide Synthesis Laboratory (University of British Columbia, Vancouver, Canada). A total of 40 Operon (Operon Technologies) and 699 University of British Columbia (Carlson) arbitrary nucleotide sequences were used in this analysis. Map positions of RAPD (and subsequently AFLP) polymorphisms were initially positioned via a low-resolution interval-mapping population, followed by all the recombinants between *Xbcd249.1* and *Xmwg036* in the high-resolution mapping population.

PCR amplification was performed in a 25-μl reaction volume with a 1× reaction buffer supplied by the manufacturer [20 mm Tris-HCl (pH 8.4), 50 mm KCl], 1.5 mm $MgCl_2$, 0.001% gelatin, 0.1 mm each of dNTP, either 5 μm decamer RAPD- or 20 μm STS-primer, 50 ng of genomic DNA, and 0.625 units of *Taq* DNA polymerase (GIBCO BRL, Rockville, MD). The following programs were used for amplification: for RAPD, one cycle for 1 min at 94°; 44 cycles for 5 sec at 94°, 30 sec at 36°, 1 min at 72°, with a final extension of 9 min at 72°; for the STS analysis, one cycle for 3 min at 94°; 29 cycles for 30 sec at 94°, 1 min at 60°, 1 min at 72°, with a final extension of 4 min at 72°. All PCR amplifications were performed in a PTC-100 programmable thermocycler (MJ Research Inc., Watertown, MA). Amplification products were resolved by electrophoresis at 80 V for 4 hr on a 2% thin (3-mm) agarose gel containing 1× TBE buffer (0.089 m Tris, 0.089 m Borate, 0.002 m Na_2EDTA; Sambrook *et al.* 1989) and 1 μg/ml ethidium bromide.

Cloning of polymorphic RAPD fragments: DNA fragments were isolated by extracting an agarose plug with the small end of a pasteur pipette followed by placement in 100 μl sterile double-distilled water (ddH_2O) to elute overnight at 4°. One microliter of eluted DNA/ddH_2O solution was used as a template for reamplification with the original 10-base oligonucleotide primer. DNA inserts were purified via a modified NA45 membrane (Schleicher & Schuell, Keene, NH) extraction, ligated into pGEM-T (Promega, Madison, WI), and transformed into the *Escherichia coli* TB-1 host strain.

AFLP analysis: All 256 pairwise combinations of ^{33}P-labeled (New England Nuclear Life Science Products, Boston, MA) *Eco*RI and *Mse*I primers (listed in Table 1) were used to screen for polymorphisms between both the pools and the parents. AFLP analysis was performed as per the AFLP instruction manual (GIBCO BRL). For each pool, 3 μl of the preamplified products from each of the 16 individual lines was combined, diluted 50×, and selective amplification was carried out in the presence of [^{33}P-γ]ATP-labeled *Eco*RI primer and *Mse*I primer (as shown in Table 1). The amplified fragments were size-fractionated through a 7% acrylamide gel (Long Ranger; FMC Bioproducts, Rockland, ME) and exposed directly (without drying the gel) to Biomax XR film (Eastman Kodak, Rochester, NY) at −80° for 16–24 hr.

Sequence-specific AFLP: The AFLP preamplification products were obtained through the use of the E-A/M-C, E-A/M-T, E-G/M-C, and E-G/M-T primer pairs. For selective amplification, the [^{33}P-γ]ATP-labeled, long terminal repeat (LTR) sequence (5′-TGTTGGAATTATGCCCTAG-3′) of the barley Bare-1-retrotransposon (Waugh *et al.* 1997) was utilized in combination with one of the random AFLP primers (listed in Table 1).

Cloning and sequencing of AFLP DNA fragments: AFLP fragments were identified by matching the target signal on the autoradiogram with its corresponding area in the acrylamide gel. The cut gel slices were dissolved in ddH_2O overnight at 4° and the fragments were enriched using only the *Eco*RI primers via 10 cycles of PCR, followed by amplification with both *Eco*RI and *Mse*I primers for another 30 cycles. The resulting fragment was cloned into pGEM-T cloning vector and transformed into the *E. coli* TB1 host strain for selection of putative clones. The cloned inserts were prescreened by direct PCR with the T7-1 (5′-AATACGACTCACTATAG-3′) and SP6 (5′-GATTTAGGTGACACTATAG-3′) primer pairs. Comigration (via polyacrylamide gel electrophoresis) of the cloned AFLP insert as compared to the original genomic AFLP fragment was used for final verification. Two confirmed colonies from each cloning experiment were purified with Microcon-100 (Amicon, Bedford, MA) and sequenced using T7-2 (5′-CGACTCACTATAGGGCGAAT-3′) and SP6-2 (5′-GCGTTG GGAGCTCTCCCATATGGT-3′) vector primers. DNA sequenc-

TABLE 1

AFLP primers used for bulk segregant analysis

	Source		
		This study[b]	
Primer[a]	GIBCO BRL[c]	Standard AFLP[c]	Sequence-specific AFLP
*Eco*RI series	AAC, AAG, ACA, ACC	AAA, AAT, AGA, AGT	GAA, GAC, GAG, GAT
	ACG, ACT, AGC, AGG	ATA, ATC, ATG, ATT	GTA, GTC, GTG, GTT
*Mse*I series	CAA, CAC, CAG, CAT	CCA, CCC, CCG, CCT	TCA, TCC, TCG, TCT
	CTA, CTC, CTG, CTT	CGA, CGC, CGG, CGT	TGA, TGC, TGG, TGT

[a] Designates the three-nucleotide sequence added to the 3′ end of the core sequence. The core sequence of *Eco*RI series is 5′-AGACTGCGTACCAATTC-3′ and the core sequence of *Mse*I series is 5′-GATGAGTCCTGAG TAA-3′.

[b] Primers other than those supplied by GIBCO BRL were designed by the Wise lab and synthesized by the ISU DNA Synthesis and Sequencing Facility.

[c] Primers for both standard and sequence-specific AFLP.

ing and oligonucleotide synthesis were performed by the Iowa State University DNA sequencing and synthesis facility. PCR primers were designed according to the DNA sequences of the clones with the assistance of Oligo 5.0 software (PE Biosystems, Foster City, CA).

Preparation of chromosomal yeast/YAC DNA: YAC clones were grown and maintained using Kiwi media (Ausubel *et al.* 1988). The AB1380 yeast host strain was grown and maintained using YEPD media [1% (w/v) bacto-yeast extract, 2% (w/v) bacto-peptone, 2% (w/v) glucose, 100 mg/l adenine]. Single-colony purified YAC clones were used to inoculate 25 ml of selective media and grown for 24–36 hr with shaking at 30°. Cells were harvested (5000 × *g*, 10 min at 4°) and resuspended in 5 ml 50 mm Na_2EDTA (pH 8.0). Yeast cell concentration was determined with a hemacytometer. Subsequently, cells were harvested and resuspended at a concentration of 1×10^9 cells/ml in resuspension buffer (10 mm Tris, pH 7.2, 20 mm NaCl, 50 mm Na_2EDTA). The cell suspension was prewarmed to 50° briefly before adding lyticase (Sigma, St. Louis) to 1 mg/ml and gently mixing with prewarmed InCert agarose [FMC Bioproducts; 2% (w/v) in resuspension buffer], and removed to plug molds. Plugs were allowed to set for 10 min at 4° before being removed from the molds into 5 ml of lyticase buffer (10 mm Tris, pH 7.2, 50 mm Na_2EDTA, 1 mg/ml lyticase) per ml of plug and incubated for 1 hr at 37°. Plugs were washed once (10 min at RT) in 1× wash buffer (20 mm Tris, pH 8.0, 50 mm Na_2EDTA) before being transferred to 5 ml of proteinase K reaction buffer [100 mm Na_2EDTA, pH 8.8, 0.2% (w/v) sodium deoxycholate, 1% (w/v) sodium lauryl sarcosine, 1 mg/ml proteinase K] per ml plug and incubated for 48 hr at 50°. Plugs were washed three times (10 ml per ml of plug; 30 min at RT) in 1× wash buffer (1 mm PMSF was included in the second wash to eliminate residual proteinase K) before a final wash in 0.1× wash buffer and storage at 4°.

Pulsed-field gel electrophoresis analysis of YAC and BAC clones: Pulsed-field gel electrophoresis (PFGE) conditions were as calculated by the auto-algorithm function of a Bio-Rad (Hercules, CA) Chef Mapper XA. The YAC and BAC clones were resolved by PFGE at 14° using 0.5× TBE buffer and 1% SeaKem LE agarose (FMC Bioproducts). PFGs were stained with ethidium bromide (1 μg/ml in ddH_2O), UV nicked (60 mJ/cm^2), and DNA transferred to Hybond N^+ membrane (Amersham Pharmacia Biotech, Piscataway, NJ) using 1.5 m NaCl, 0.4 m NaOH as the transfer buffer. YAC clones were further restriction mapped utilizing a partial digest strategy described by Burke *et al.* (1987), employing medium-rare and rare-cutting restriction endonucleases.

Generation of hybridization probes for the physical mapping of YACs: Sequences specific for the right and left arms of the YAC vector were amplified via PCR using pBR322 as a template. The primers used were as follows: YAC-LA-as (5′-AAGCGAGCAGGACTGGGCGG-3′) in conjunction with YAC-LA-s (5′-GTCAGCGGGTGTTGGCGGGT-3′) amplified a 2.5-kb product specific for the left arm of the YAC vector. YAC-RA-as (5′-CGGTTTTTTCCTGTTTGGCT-3′) in conjunction with YAC-RA-s (5′-TTGTTTCGGCGTGGGTATGG-3′) amplified a 1.4-kb product specific for the right arm of the YAC vector. Amplification conditions for the PTC-100 programmable thermal cycler (MJ Research) were as follows: one cycle for 3 min at 94°; 34 cycles for 30 sec at 94°, 30 sec at 56°, 3 min at 72°; and one final extension cycle for 10 min at 72°.

Genetic mapping of YAC ends or other sequences derived from the *Mla* region: Barley DNA was isolated from frozen tissue using a modified CTAB extraction. These DNA extractions, as well as DNA gel blot analyses, were conducted as previously described (Wise and Schnable 1994). YAC ends or other sequences derived from the *Mla* region were first established as being low-copy by hybridization with strip blots of *Hin*dIII digested and resolved parental DNA. Once low-copy status had been determined, sequences were screened for RFLPs by Southern hybridization with parental DNA digested with a number of restriction enzymes to reveal which restriction endonuclease revealed a polymorphism. RFLPs were exhibited as differences between the two parental lines and were mapped by Southern hybridization with recombinants from our high-resolution mapping population (Manley 1993).

BAC AFLP fingerprinting: BAC DNA was isolated by using the Clemson University Genomic Institute (CUGI; 1998) protocol. The standard AFLP analysis protocol was followed except that the preamplification and amplification primer pairs are identical. These primers do not contain a selective base. The sequence of the *Eco*RI primer (designated E-0) is 5′-AGACTGCGTACCAATTC-3′ and the sequence of the *Mse*I primer (designated M-0) is 5′-GATGAGTCCTGAGTAA-3′.

BAC-end cloning and sequencing: BAC ends were cloned via double-end rescue. Briefly, the BAC DNA (0.5 μg) was digested for 4 hr at 37° with 20 units of *Nsi*I (New England Biolabs, Beverly, MA), which does not cut within the vector, but cuts fairly frequently in the genomic-DNA insert. The reaction was inactivated for 20 min at 70°. The BAC DNAs were recircularized by self-ligation in 200 μl overnight at 16°.

The ligated products were transformed into the *E. coli* TB1 host strain and plated on LB-chloramphenicol plates. For sequencing, "mini-BAC" DNA was prepared according to our standard BAC purification protocol and further concentrated through Microcon-100 columns (Amicon, Beverly, MA). Sequence data were obtained with ABI Big Dye terminators (PE Biosystems) using the T7-1 or M13 reverse (designated R1; 5′-GGAAACAGCTATGACCATG-3′), using 5 μg of total template DNA. The resulting sequence data were utilized for designing PCR primers for mapping and further library screening.

Low-pass BAC sequencing: Cesium chloride-density gradient purified BAC DNA was used for sequencing library construction. Two milliliters of BAC DNA solution [20 μg DNA, 500 μl glycerol, 200 μl 10× TM (0.5 m Tris-Cl, 150 mm $MgCl_2$)] was nebulized with N_2 at 6 psi for 2 min. DNA fragments of 1.7–3.0 kb were agarose-gel purified and ligated to dephosphorylated *Sma*I-restricted pUC18 (Boehringer Mannheim, Indianapolis) overnight at 16°. One microliter of the ligation solution was transformed into 25 μl of *E. coli* electroMAX DH10B TM competent cells (GIBCO BRL) by electroporation with the Cell-Porator system (GIBCO BRL; 400 V, capacitance 330 μF, impedance low ohms, charge rate fast, voltage booster resistance 4 kohms). White colonies were picked into 96-well microtiter plates containing LB freezing medium and ampicillin, incubated overnight at 37°, and stored at −80°.

RESULTS

Four *Mla* specificities are inseparable by recombination in the C.I. 16151 × C.I. 16155 cross: Over 30 specificities of *Mla* have been described (reviewed by Jørgensen 1992, 1994; Kintzios *et al.* 1995). Two of these specificities are present in coupling in each of our mapping parents. The Franger-derived line, C.I. 16151, contains the *Mla6* and *Mla14* specificities as described previously (Giese *et al.* 1981; Jørgensen 1992, 1994). Likewise, the Rupee-derived line, C.I. 16155, contains the *Mla13* and *Ml-Ru3* specificities (Jørgensen 1992, 1994; Caffier *et al.* 1996). Previously, we determined the position of the *Mla6*, *Mla13*, and *Mla14* specificities (Mahadevappa *et al.* 1994). The first objective of this study was to confirm the position of *Mla14* and to determine the position of the fourth specificity in our mapping population, *Ml-Ru3*.

Unique ITs in response to characterized isolates of *E. graminis* are utilized to detect the different specificities in segregating populations. In our earlier studies, we had mapped the *Mla14* specificity via inoculation of the recombinant population with isolate A27 (Mahadevappa *et al.* 1994). As shown in Table 2, the C.I. 16151 line that contains *Mla14* confers an IT of 2–3n in response to isolate A27, whereas the C.I. 16155 line containing *Mla13* imparts an IT of 0 with the same isolate. However, the 0 IT in response to *Mla13* would be predicted to be epistatic over the 2–3n IT displayed by *Mla14*. Thus, it was conceivable that some of the recombinant progeny in our segregating population would display a 0 IT in response to *Mla13*, but still contain *Mla14*. This epistasis could have complicated mapping of the *Mla14* specificity.

To map the position of *Mla14* and *Ml-Ru3*, 88 homozygous families that are recombinant between *Xbcd249.1* and *Xmwg036* were utilized. Four to six progeny from each homozygous recombinant family were inoculated separately with the R63, R189, A27, and 5874 isolates of *E. graminis* (shown in Table 2) and subsequently scored for IT using a 0–4 scale. Isolate R63 imparts a unique IT only in response to *Mla14*; thus, screening with this isolate would provide an unambiguous result. First, the 49 recombinant individuals that displayed an IT of 0 in response to A27 (confirming the presence of *Mla13*) also displayed an IT of 4 in response to R63, indicating that these individuals, in fact, do not contain *Mla14*. Conversely, the remaining 39 recombinant individuals that displayed an IT of 2–3n in response to A27 also displayed the identical IT in response to R63. This confirmed the presence of *Mla14* in these 39 progeny. Therefore, these additional results from the inoculations with R63 confirmed that *Mla14* specificity cosegregates (in repulsion) with *Mla13*.

To position *Ml-Ru3*, the recombinant population was inoculated with isolate R189. The C.I. 16155 parent that contains *Ml-Ru3* displays an IT of 1–2n in response to this isolate. Forty-nine recombinant individuals dis-

TABLE 2

Infection type of recombinant lines in the *Xbcd249.1-Xmwg036* interval 7 days after inoculation with *E. graminis* f. sp. *hordei*

E. graminis isolate	Host specificity[a]				Number of plants with infection type				
	Mla6 (C.I. 16151)	*Mla14* (C.I. 16151)	*Mla13* (C.I. 16155)	*Ml-Ru3* (C.I. 16155)	0–1	1–2	2–3n	4	Total
A27	—	2–3n	0	—	49[b]	0	39[c]	0	88
R63	—	2–3n	4	4	0	0	39[c]	49	88
R189	—	2–3n	—	1–2n	0	49[d]	39[c]	0	88
5874	0	—	4	4	39[e]	0	0	49	88

[a] Infection type (IT): 0, immune; 1n, small necrotic flecks (0.5 mm); 1–2n, small necrotic flecks (1 mm) with very limited sporulation; 2n, large necrotic flecks (1.5 mm) with no sporulation; 2–3n, large necrotic flecks (1.5 mm) with limited sporulation; 4, abundant sporulation. —, this specificity cannot be detected with the designated isolate.

[b, c, d, e] These individuals carry *Mla13*, *Mla14*, *Ml-Ru3*, and *Mla6*, respectively.

played an IT of 1–2n in response to R189, indicating the presence of *Ml-Ru3* (Table 2). These same 49 individuals also displayed an IT of 0 in response to A27, indicating the presence of the *Mla13* specificity (in coupling). Importantly, the remaining 39 recombinant individuals that displayed an IT of 0 in response to 5874 (confirming the presence of *Mla6*), also displayed an IT of 2–3n in response to A27, R63, and R189, indicating the presence of *Mla14*. On the basis of these experiments, we established that the *Ml-Ru3* specificity cosegregates (in repulsion) with *Mla6* and *Mla14*. Thus, current observations indicate that all four specificities in this mapping population (of 3600 gametes) are at the same genetic position on chromosome 5 (1H). This observation could be viewed as advantageous, because it suggested that all four of these specificities could be physically close, which would facilitate their ultimate isolation.

We also further tested two putative recombinants that were reported previously in Mahadevappa *et al.* (1994). In that report, we had postulated recombination between the *Mla6* and *Mla13* specificities in two F_3 families of this same mapping cross. To review, both of these putative recombinant lines (H92S 6526 and H92S 6562 in Table 5 of Mahadevappa *et al.* 1994) contained one or more recombination events between the flanking markers, *Hor1* and *Hor2*, that we were using to screen the population. In addition, these progeny families displayed an IT ratio in response to infection with isolates A27 and 5874 that was consistent with a recombination event (or gene conversion) within *Mla*. However, lack of DNA markers tightly linked to *Mla* prevented the precise fingerprinting of recombination events in our previous work. Therefore, to follow up on our assumption, the putative recombinant lines H92S 6526 and H92S 6562 were subjected to several progeny tests with isolates 5874 and A27. However, when these H92S 6526 and H92S 6562 progeny were genotyped with our current tightly linked markers, the intra-*Hor1-Hor2* recombination events appeared to be positioned on either side of the *Mla* locus. Hence, even though the original lines repeatedly displayed non-Mendelian IT ratios, at present, we are unable to confirm our original hypothesis of recombination between *Mla6* and *Mla13* at the molecular level. It is possible that a distorted segregation of parental chromosomes caused the altered IT ratios in the F_3 in these two families.

Bulk-segregant, RAPD analysis increases the genetic resolution flanking the *Mla* cluster: Saturation of the target interval with DNA markers is a prerequisite for physical delimitation via large-insert clones in the complex barley genome. A 3-cM window, defined by the *Xbcd249.1–Xmwg036* interval (DeScenzo *et al.* 1994), was established from our high-resolution population for bulk segregant (Giovannoni *et al.* 1991; Michelmore *et al.* 1991; Churchill *et al.* 1993) RAPD, and AFLP (Vos *et al.* 1995) analyses.

A total of 739 RAPD primers were used to amplify DNAs from the defined bulks. Of these, 91 primers produced DNA fragments that were polymorphic between C.I. 16151 and C.I. 16155, or the bulks. Eighteen recombinant lines, each possessing a unique recombination breakpoint between the *Hor1* and *Hor2* loci, were used to quickly determine if markers were positioned near the *Mla* locus. Only 3 of the 91 primers that produced amplified polymorphisms mapped to the region between *Hor1* and *Hor2*. Primer OPA-10 (5′-GTGATCG CAG-3′) amplified a 1500-bp fragment in C.I. 16155, designated $OPA\text{-}10_{1500}$, that mapped between *Hor1* and *XChs3*. Primers UBC465 (5′-GGTCAGGGCT-3′) and UBC165 (5′-GAAGGCACTG-3′) amplified 950-bp and 1626-bp fragments, respectively, in pools containing the *Mla6* or *Mla14* specificities but not in pools containing the *Mla13* or *Ml-Ru3* specificities. The 950-bp fragment was designated $UBC465_{950}$ and mapped between *XChs3* and *Xmwg068*. The 1626-bp fragment was designated $UBC165_{1626}$ and cosegregated with *Mla* in the low-resolution, interval-mapping population described above.

Eighty-eight lines, each containing a unique recombination breakpoint in the *Xbcd249.1–Xmwg036* interval, were used to fine-map $UBC165_{1626}$ to a position 0.3 cM proximal to *Mla6*. The 1626-bp $UBC165_{1626}$-derived fragment was subsequently cloned, sequenced, and a series of PCR primers were designed. The different pairwise combinations yielded a number of genomic-PCR products, which is likely due to the repetitive sequence represented by the 1626-bp $UBC165_{1626}$-derived fragment. The combination of primers P0 (5′-GAAGGCACTGAATCG TTGATGG-3′) and P954RC (5′-CAGTTTAGGGAAG TATTGCATC-3′) produced a C.I. 16151-specific product that mapped 0.28 cM distal to *Mla*. Apparently, the primer pair P0 and P954RC uncovered a sequence-related, tightly linked copy of $UBC165_{1626}$. This amplification product consistently yielded the most stable map

TABLE 3

AFLP markers tightly linked to the *Mla* locus

Marker designation	Primer pair[a]		Position[b]	STS marker developed
	*Eco*RI	*Mse*I		
FW36.4	AGG	CTA	1.27 D	No
FW36.2	AGC	CTG	1.24 D	No
FW26.7	AGG	CTA	1.15 D	No
FW56.0	AAC	CTT	0.65 D	No
FW108	ACG	CAT	0.14 D	Yes
FW16.8	AAC	CAG	0.59 P	No
FW15.4	ACG	CAG	0.80 P	No

[a] The three-letter sequence designates the three selective bases added to the 3′ end of the core sequence. The core sequence for *Eco*RI primers is 5′-AGACTGCGTACCAA TTC-3′ and the core sequence for *Mse*I primers is 5′-GATGA GTCCTGAGTAA-3′.

[b] Distance in centimorgans distal (D) or proximal (P) to the *Mla* locus.

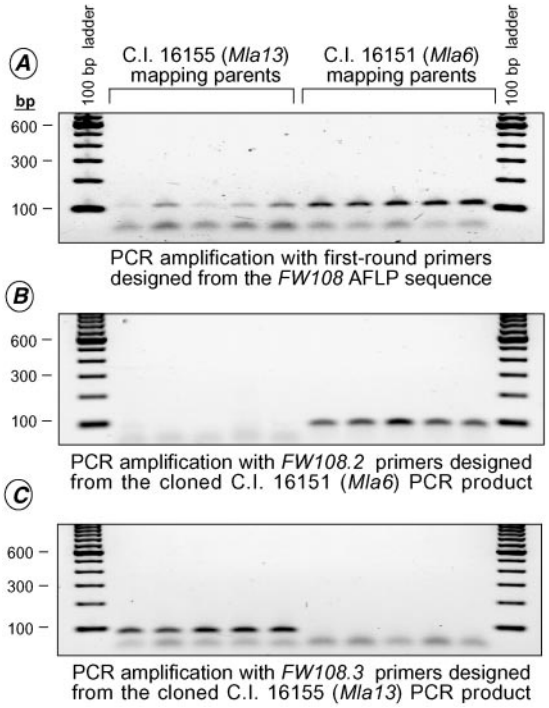


Figure 1.—PCR products amplified from C.I. 16155 and C.I. 16151 parental DNAs with *FW108* allele-specific primers. (A) Parental-specific PCR products generated from first-round PCR primers designed according to the DNA sequence of the AFLP clone. (B) C.I. 16151-specific PCR products generated from *FW108.2* primers. (C) C.I. 16155-specific PCR products generated from *FW108.3* primers. The *FW108.2* primers were used to screen the Maltagen (Franka) YAC library.

position and was designated *Fr1062*. The *Fr1062* PCR primers amplified the same fragment from Franka, the cultivar used in the construction of the Maltagen YAC library (Kleine *et al.* 1993, 1997).

AFLP analysis is used to further saturate the genetic map: To further enrich the *Mla* region with markers for large-insert clone isolation, 256 AFLP primer pairs were used to screen for polymorphisms between the C.I. 16151 and C.I. 16155 mapping parents and the pools described above. Out of 22,500 AFLP fragments generated, 132 polymorphisms amplified from 104 primer pairs were observed. Seven of these polymorphic fragments mapped to the *Xbcd249.1–Xmwg036* interval on our low-resolution, interval-mapping population. In the high-resolution analysis (Table 3), it was established that the *FW108* AFLP marker was 0.14 cM distal from the *Mla* locus and allele-specific primers were developed for library screening as described below.

Development of allele-specific, AFLP-derived STS markers: First-round PCR primers were designed according to the corresponding DNA sequence of the cloned AFLP fragment. Six of the primer pairs derived from the internal sequences of the seven markers did not display a polymorphism in amplification experiments of the parental DNAs (Table 3). However, the *FW108*-derived marker displayed a potential polymorphism, amplifying a strong band in parental DNA from C.I. 16151 but a weak band in parental DNA from C.I. 16155; this same pattern was observed among the recombinants in the high-resolution mapping population.

The *FW108* PCR products from both the C.I. 16151 and C.I. 16155 parental DNAs (Figure 1A) were cloned and sequenced. Three single-nucleotide polymorphisms (SNPs) were detected in the 108-bp *FW108* fragment (Figure 2). These SNPs facilitated the design of C.I. 16151- and C.I. 16155-specific forward primers (designated *FW108.2* and *FW108.3*, respectively). As illustrated in Figure 1, it was established that when paired with *FW108* reverse primer, the *FW108.2* and *FW108.3* primers amplified allele-specific polymorphisms that mapped to the site of the original AFLP marker, *FW108*. This approach was not useful for the development of STS primers for the other six AFLP markers. The original *Eco*RI/*Mse*I polymorphism was lost when the amplified fragments were cloned and the internal sequence of the C.I. 16151 and C.I. 16155 parental fragments were 100% identical.

Bare-1 retrotransposon, sequence-specific AFLP is used to identify additional markers: Sequence-specific AFLP (S-SAP) was utilized to further screen for markers close to the *Mla* cluster. There are at least 3×10^4 copies of the Bare-1 retrotransposon in barley, which is equivalent to 6.7% of the genome (Suoniemi *et al.* 1996). Therefore, the Bare-1 inverted repeat primer was used in conjunction with 24 *Eco*RI- and 24 *Mse*I-primers to amplify DNAs from the bulks and the parents. Two separate preamplifications were used for a total of 96

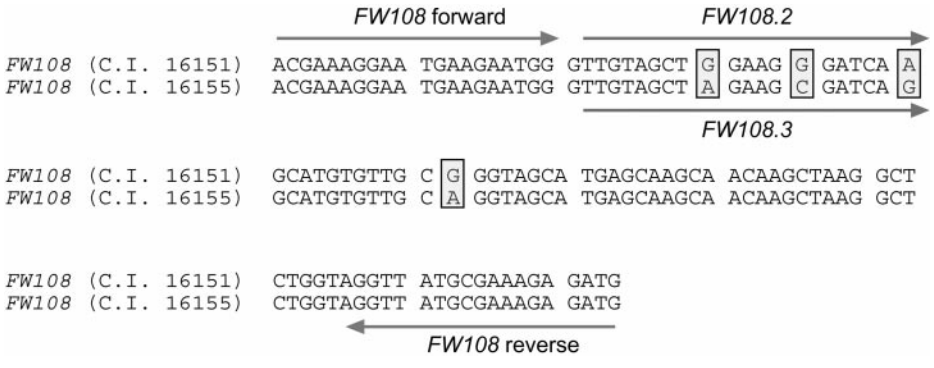


Figure 2.—Nucleotide sequence of the *FW108* fragment from C.I. 16151 and C.I. 16155. The allele-specific primers *FW108.2* and *FW108.3* were designed on the basis of the three-nucleotide sequence polymorphism as shown.

pairwise combinations. From the resulting 5700 amplified bands, 114 polymorphisms were detected. One DNA fragment, designated *AGCBare*, cosegregated with the map position of *Fr1062*, 0.28 cM distal to the *Mla* locus. The number of polymorphisms detected in the bulks suggests that there are multiple, near-identical copies of the Bare-1 retrotransposon in the *Mla*-flanking region.

The *236R* end clone from YAC236 cosegregates with the *Mla* cluster: Figure 3 illustrates the integration of all new RAPD, AFLP, and derived STS markers into the *Hor1-Mla-Hor2* region of chromosome 5 (1H). The *Fr1062*- and *FW108.2*-derived primers amplified DNA from Franka, and thus, markers fulfilled the criteria for large-insert clone isolation. Therefore, these two primer sets were used to screen the Maltagen (Franka) YAC library (Table 4; Kleine *et al.* 1993, 1997). As shown in Figure 4, YAC clones were sized by PFGE followed by Southern hybridization with YAC vector-specific sequences. YAC terminal-end sequences were isolated by inverse PCR (Leister *et al.* 1997b). It was established that two of these ends (*234L* and *236R*) hybridized to low-copy fragments that were polymorphic between the

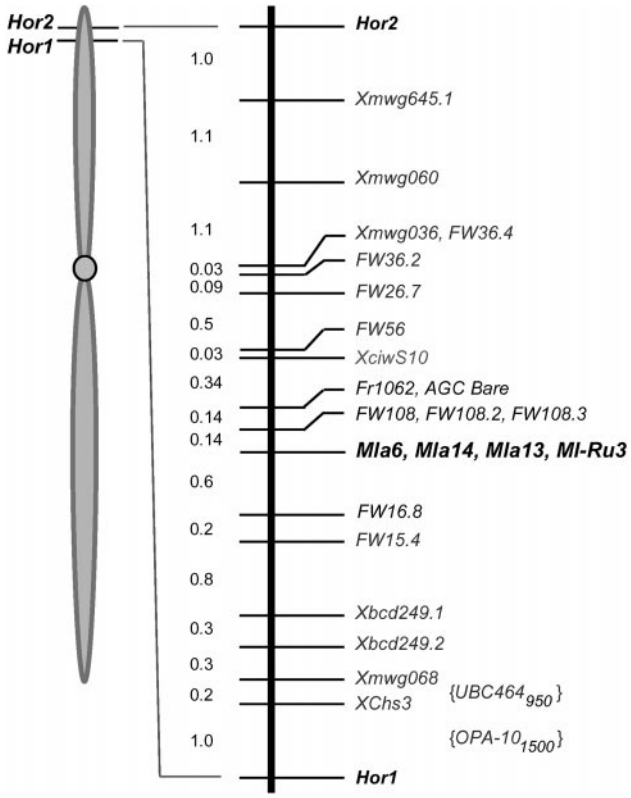


Figure 3.—High-resolution genetic map of the *Hor1–Hor2* region of barley chromosome 5 (1H). *Fr* and *FW* prefixes designate Wise laboratory RAPD- and AFLP-derived markers, respectively. Markers in brackets were mapped on the low-resolution interval population. An *X* prefix designates an RFLP marker; *mwg* markers are from Munich-Weihenstephan-Grunbach; *bcd* markers are from Cornell University; and *ciw* markers are from the Carnegie Institute of Washington.

TABLE 4

YAC clones isolated from the Maltagen Franka library

Clone designation[a]	Identified by[c]	Insert size (kb)	Primers for right end[b] (5′–3′)	Primers for left end[b] (5′–3′)	Annealing temperature
75	*FW108.2*	200	AGGGCACTCTCAGGGCACTGG TCCGGATGTTCCTTGTGGTAACAC		56
101	*Fr1062*, *FW108.2*	50			
105	*FW108.2*	30	GGATCGGCGAATTCCGTATG GAATTCAAACAGATTCATAA		56
152	*FW108.2*	30/200			
234	*FW108.2*	50		TGATGTCGCGATGTTGCTCG GAATTCTGCTTGAAACCATT	56
236	*Fr1062*, *FW108.2*	280/600	CTCCTCACATCGGCTAGCTAGAT AAACCAATTAAGAGGCAGTGCAT	GAATTCTGCAGCGTTGTCCATT CCTGGCATGATCGCCTGGGCCAT	56
98IIF5	*mwg2197*, 236R	160			
99IIE7	*mwg2197*, 236R	160			
120ID1	*mwg2197*, *236R*	170			

[a] YAC clones were identified by PCR amplification with *Mla* region markers.
[b] Primers used to amplify the ends of the designated YAC.
[c] RFLP, RAPD, and AFLP markers used to identify various YACs.

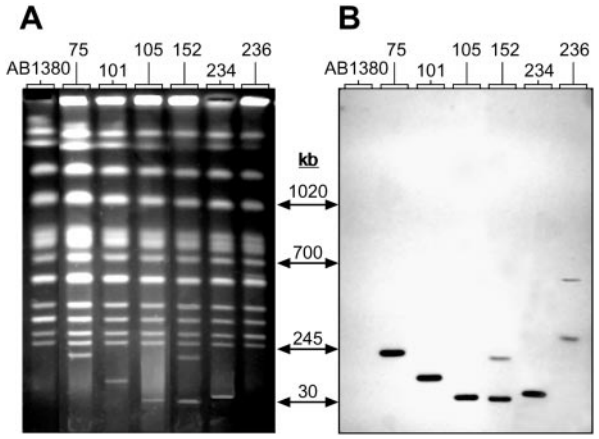


Figure 4.—Analysis of *Mla*-linked YAC clones. High-resolution genetic analyses established that the *Fr1062*-RAPD- and *FW108*-AFLP-derived markers were 0.28 and 0.14 cM distal to the *Mla* locus, respectively, and allele-specific primers were developed to screen the Maltagen (Franka) YAC library. (A) YAC clones were resolved by PFGE using a CHEF Mapper XA system (Bio-Rad) in conjunction with a 14-cm gel. (B) Southern analysis of YAC clones. Filters were then hybridized with YAC vector-specific sequences to identify all YACs.

C.I. 16151 and C.I. 16155 mapping parents. Subsequently, a combination of genetic and physical mapping established that one of the two copies of *236R* cosegregates with the *Mla* locus. During the course of this investigation, we also mapped the RFLP markers, *mwg2083* and *mwg2197*, previously shown to map between the *Hor1* and *Hor2* loci (http://wheat.pw.usda.gov/ggpages/maps.html; kindly provided by Dr. Andreas Graner, IPK, Gatersleben, Germany). One of the three copies of *mwg2083* cosegregated with the *Mla* locus. The single-copy marker *mwg2197* was positioned two crossovers distal (0.056 cM) to the *Mla* locus. We also positioned the *RGH Hv-b6.1* (Leister *et al.* 1997a). We hypothesized that by taking this candidate-gene approach, we might identify large-insert clones that contained the *Mla* gene family. This proved not to be the case as *Hv-b6.1* cosegregated with *XciwS10*, which is 0.62 cM distal to the four specificities in our mapping population (see Figure 5).

Additional overlapping YACs are identified with primers developed from *236R*: For the first step in our chromosome walk to span the *Mla* cluster, primers were developed from the sequence of *236R* and *mwg2197* and used to identify the three overlapping Franka YACs, 98IIF5, 99IIE7, and 120ID1 (Table 4). YACs 98IIF5, 99IIE7, and 120ID1 were mapped physically via a partial digest strategy. This physical analysis indicated that YACs 98IIF5 and 99IIE7 contained identical DNA inserts but were cloned in opposite orientations. YAC 120ID1 is identical to YACs 98IIF5 and 99IIE7 except for an additional ~10 kb that begins ~14 kb from the *mwg2197* end of this YAC. It is likely that these three YACs correspond to YAC2197 A, B, and C reported by Schwarz *et al.* (1999), as they were isolated from the same library (Kleine *et al.* 1997) with primers developed from *mwg2197*.

DNA gel blots of total YAC digests were hybridized with probes *236R*, *mwg2197*, and *mwg2083* to physically position them on the contig. It was established that *236R* and *mwg2083* both lie within the same ~7-kb subregion located ~12 kb from the left end of YAC 120ID1. Similarly, it was determined that *mwg2197* lies on a ~7-kb subregion positioned ~14 kb from the right end of YAC 120ID1 (Figure 5). These restriction analyses indicated that the physical distance between *236R*/*mwg2083* and *mwg2197* is ~120 kb. In preparation for sequencing, YAC 120ID1 was further fractionated to create a subgenomic pBeloBAC11 library. The identified Franka BACs IV16.11, I6.24, I3.2, and VI12.7 all hybridize to *236R* and Franka BAC III12.9 hybridizes to *mwg2197*.

Overlapping Morex BACs are identified to extend the physical contig: Unfortunately, no additional YACs could be identified that allowed us to extend the Franka contig proximal to *236R*. Therefore, for the next step in our chromosome walk, amplified products from *236R*, *234L*, *mwg2083*, and *mwg2197*, were used as hybridization probes on high-density filters of a new 6.3-genome-equivalent BAC library from the barley cultivar Morex [Clemson University Genomic Institute (CUGI)]. The *Mla*-cosegregating markers *236R* and *mwg2083* each hybridized to three classes of Morex BACs. These classes most likely originated from different regions of the genome and were designated class I (typified by 80H14, shown in Table 5), class II (typified by 192H7, not shown), and class III (not typified by 80H14 or 192H7, not shown). To determine which class of Morex BACs overlapped with the Franka YACs, a number of approaches were employed. First, representative members of the three classes of Morex BACs and the five YAC

Figure 5.—Genetic and physical map of the *Mla* region. This comparison of physical to genetic distance in the *Mla* region was obtained by the use of common probes/primers on our high-resolution mapping population in addition to the overlapping Franka YACs and Morex BACs. Franka YACs are designated by a "Fr" prefix and are shown in dark red, whereas Morex BACs are designated only by their library addresses and are shown in black. A vertical red rectangle designates a cloned end-sequence from which the primers in Table 6 were developed and subsequently used for genetic and/or physical mapping. An orange filled-in circle designates that YAC/BAC was amplified by the respective end-clone primer set or it hybridized to the amplified product. An X under the top horizontal line represent crossovers in the recombinant mapping population. When BAC ends were sequenced, horizontal arrowheads designate the T7 side of the vector. Distances are in centimorgans across the top horizontal line and YACs/BACs in the 1080-kb contig are drawn to scale in kilobases below.

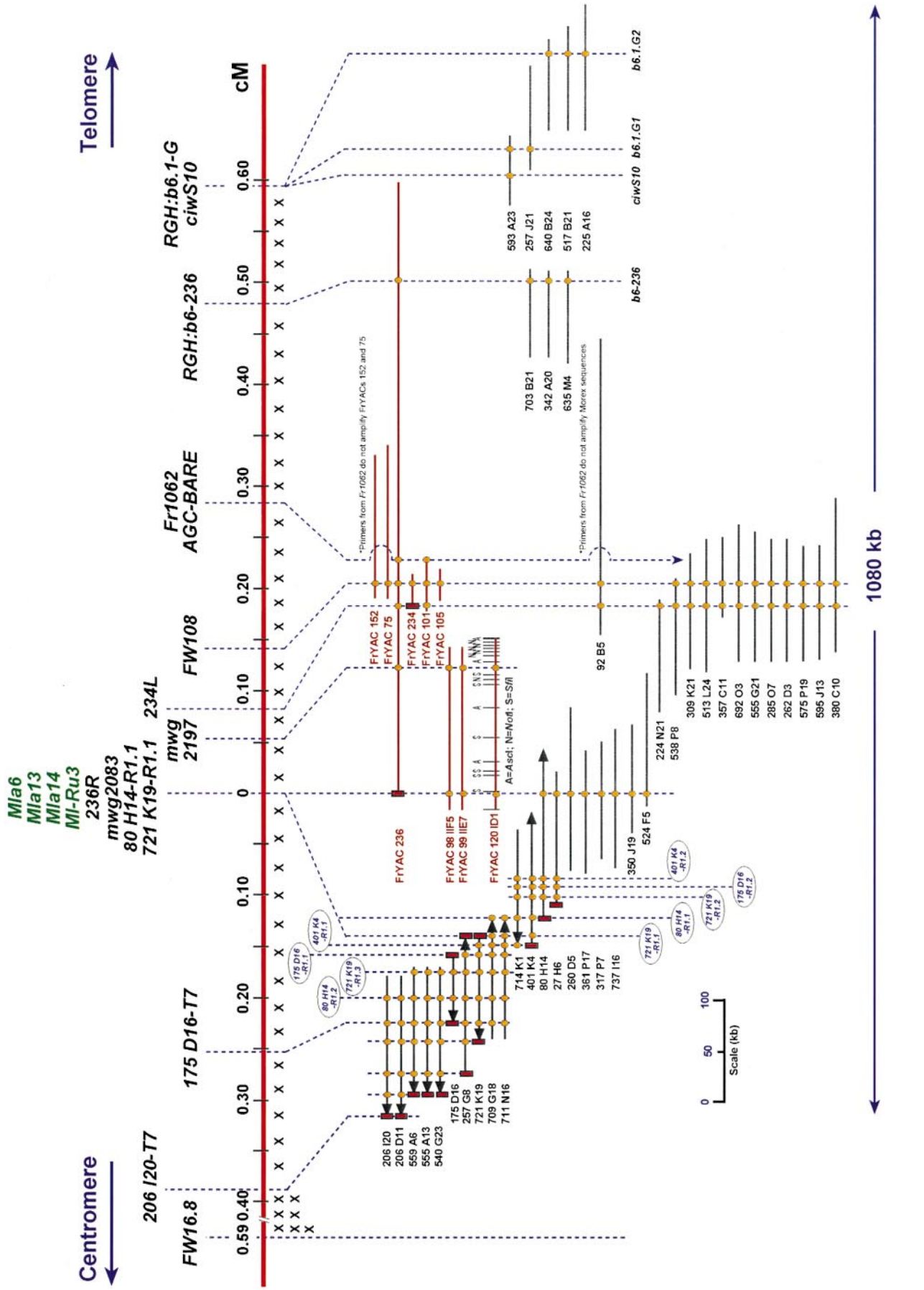

Telomere
Centromere
cM
0.59 0.40
0.30
0.20
0.10
0
0.10
0.20
0.30
0.40
0.50
0.60
FW16.8
206 I20-T7
175 D16-T7
mwg 2197
FW108
Fr1062 AGC-BARE
RGH:b6-236
RGH:b6.1-G ciwS10
234L
721 K19-R1.1
80 H14-R1.1
mwg2083
236R
Mla6
Mla13
Mla14
Ml-Ru3
b6-236
ciwS10 b6.1.G1
b6.1.G2
1080 kb
Scale (kb)
0
50
100
A=AscI; N=NotI; S=SfiI
*Primers from Fr1062 do not amplify FrYACs 152 and 75
*Primers from Fr1062 do not amplify Morex sequences
FrYAC 152
FrYAC 75
FrYAC 234
FrYAC 101
FrYAC 105
FrYAC 236
FrYAC 98 IIF5
FrYAC 99 IIE7
FrYAC 120 ID1
206 I20
206 D11
559 A6
555 A13
540 G23
175 D16
257 G8
721 K19
709 G18
711 N16
714 K1
401 K4
80 H14
27 H6
260 D5
361 P17
317 P7
737 I16
350 J19
524 F5
224 N21
538 P8
92 B5
309 K21
513 L24
357 C11
692 O3
555 G21
285 O7
262 D3
575 P19
595 J13
380 C10
703 B21
342 A20
635 M4
593 A23
257 J21
640 B24
517 B21
225 A16
401 K4 -R1.1
175 D16 -R1.1
80 H14 -R1.2
721 K19 -R1.3
721 K19 -R1.1
80 H14 -R1.1
401 K4 -R1.2
721 K19 -R1.2
175 D16 -R1.2

TABLE 5

Contiguous class I BAC clones isolated from the CUGI Morex library with *Mla*-spanning probes

Clone designation	Hybridization probe	Estimated size (kb)	Clone designation	Hybridization probe	Estimated size (kb)
524 F5	*236R, mwg2083*	133	224 N21	*234L*	108
737 I16	*236R, mwg2083*	138	538 P8	*234L*	113
317 P7	*236R, mwg2083*	117	309 K21	*234L*	111
350 J19	*236R, mwg2083*	105	513 L24	*234L*	133
361 P17	*236R, mwg2083*	120	357 C11	*234L*	80
260 D5	*236R, mwg2083*	163	692 O3	*234L*	132
27 H6	*236R, mwg2083*	130	555 G21	*234L*	130
80 H14	*236R, mwg2083*	165	285 O7	*234L*	120
401 K4	*80 H14-R1*	130	262 D3	*234L*	120
714 K1	*80 H14-R1*	115	575 P19	*234L*	122
711 N16	*80 H14-R1*	125	595 J13	*234L*	120
709 G18	*80 H14-R1*	125	380 C10	*234L*	152
721 K19	*80 H14-R1*	105	92 B5	*234L*	290
257 G8	*80 H14-R1*	140	342 A20	*B6-236*	83
175 D16	*80 H14-R1*	65	635 M4	*B6-236*	87
540 G23	*80 H14-R1*	122	703 B21	*B6-236*	86
555 A13	*80 H14-R1*	122	225 A16	*B6-236*	106
559 A6	*80 H14-R1*	122	517 B21	*B6-236*	92
206 D11	*80 H14-R1*	135	640 B24	*B6-236*	89
206 I20	*80 H14-R1*	135	257 J21	*B6-236*	94
			593 A23	*B6-236*	71

120ID1-derived Franka BACs were digested with *Hin*dIII and *Eco*RI and the resulting DNAs were size fractionated via agarose-gel electrophoresis. Due to the sequence diversity between Morex and Franka, we were unable to visually determine the overlap via comigrating *Eco*RI and *Hin*dIII restriction fragments. When a class I or class II Morex BAC was used as a hybridization probe, it appeared that class I Morex BACs were more related to the Franka BACs. However, the frequency of repetitive sequences in the barley genome complicated the interpretation. Hence, we employed a BAC AFLP fingerprinting strategy to identify small, comigrating, amplified DNA fragments. We reasoned that comigrating amplified fragments would be sequence-related and would facilitate the identification of the overlapping region between the Franka and Morex BACs. Indeed, four comigrating AFLPs of 275, 281, 595, and 693 bp were observed among class I (80H14-like) BACs and the YAC 120ID1-derived Franka BACs. Sequence analysis of these comigrating DNA fragments revealed that the class I Morex BACs were 97–98% identical to the respective sequences from the Franka BACs. Furthermore, when the low-copy 693-bp AFLP-derived fragment was used to hybridize the initial DNA gel blots described above, only class I Morex BACs and the YAC 120ID1-derived Franka BACs showed any detectable signal. In contrast, comigrating AFLPs were not detected between class II and class III from Morex and any of the YAC 120ID1-derived BACs. These results indicated that Morex class I BACs shown in Table 5, in fact, overlapped with the YAC 120ID1-derived Franka BACs.

Additional overlapping Morex BACs are identified that physically encompass the *Mla* cluster: For the third step in our chromosome walk, a low-copy probe developed from the 80H14-R1 end was used to identify 12 additional BACs from the Morex library. These 12 BACs all overlapped physically due to the existence of a second copy (80H14-R1.2; see encircled markers in Figure 5) ~50 kb proximal to the actual R1 end of 80H14. The *Mlu*I fingerprint of these BACs shown in Figure 6 illustrates the overlapping pattern and extension of the *Mla*-spanning contig.

BAC-end sequences were used to develop primers (shown in Table 6) for genetic mapping on our high-resolution population. If an amplification polymorphism was detected with the first-round primers between our C.I. 16151 and C.I. 16155 mapping parents, then these same primers were used for mapping on every recombinant between *Xmwg036* and *Xbcd249.1* (Figure 3). This approach was utilized for the *Mla*-cosegregating STS marker, *721K19-R1.1*. However, if no polymorphisms were observed but the first-round primers could be used to amplify a product from both mapping parents, then the fragments were cloned and sequenced to develop allele-specific STS primers. Three additional polymorphic markers were developed by this method. As shown in Figure 5, STS marker *80H14-R1.1* cosegregated with *Mla*, STS marker *175D16-T7* was 0.25 cM proximal to *Mla*, and STS marker *206I20-T7* was 0.40 cM proximal to *Mla*.

Additionally, individual BACs were used as template for direct PCR amplification and the products were uti-

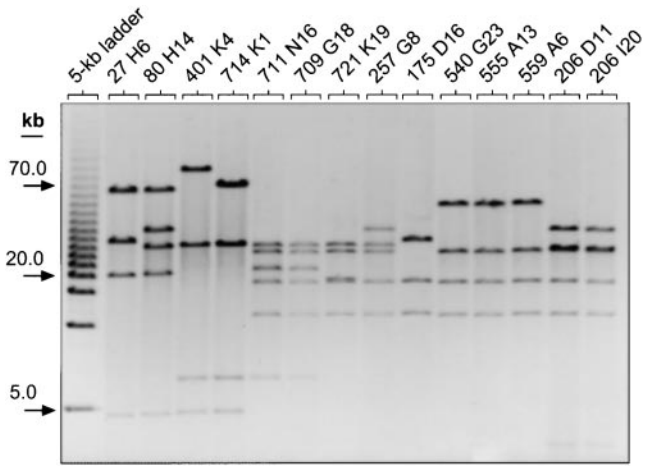


Figure 6.—*Mlu*I fingerprint analysis of BAC clones. The low-copy *236R* YAC end was used to hybridize filters of the Morex BAC library. BAC 80H14 was identified as cosegregating with the *Mla* locus and a low-copy end probe developed from 80H14 was used to rescreen the library to isolate additional BAC clones. Products of digestion were resolved by PFGE using the CHEF Mapper XA system (Bio-Rad) in conjunction with a 14-cm gel.

lized in DNA gel blot hybridization to verify the overlapping pattern. All of the primers positioned between 80H14-R1.1 and 80H14-R1.2 (Figure 5) amplified an additional fragment from BAC 80H14. This result suggests that there is a large tandem duplication of 80H14 sequences on the overlapping BACs proximal to 80H14-R1.1.

Low-pass and BAC-end sequencing reveals eight *RGH*s within a 240-kb interval: Four 96-well plates were used to sequence 384 random subclones derived from Morex BAC 80H14. These data were combined with 24 BAC-end sequences to expedite gene discovery in the *Mla*-spanning region. A total of 144,000 nucleotides from random 80H14 subclones and 13,200 nucleotides from BAC ends were utilized for computational analyses via BLASTx searches of the NCBI nonredundant (nr) database. This approach revealed eight near full-length sequences that possessed highly significant amino acid similarity to the NBS-LRR class of cloned plant-resistance genes (Ronald 1998). Seven of these sequences originated from the random sequencing of 80H14 and one was revealed by the T7-end sequencing of BAC 714K1.

Pairwise comparisons of these NBS-LRR *RGH*s were performed using BLASTn, BLASTp (Altschul *et al.* 1997), and the GAP comparison of GCG (Wisconsin Package for sequence analysis; Oxford Molecular, Madison, WI). Initially, comparisons were delimited to sequences between and including the P-loop and the "GLPLA" motif (Baker *et al.* 1997). Pairwise BLASTp comparisons of the deduced amino acid sequences indicate that these *RGH*s fall into three families. The *RGH1* family consists of five members, the *RGH2* family consists of two members, and the *RGH3* family has one member. Intrafamily GCG-GAP comparisons revealed 80–98% deduced amino acid similarity between members of *RGH* families 1 and 2, whereas interfamily comparisons showed only 46–51% amino acid similarity.

We also compared the near full-length nucleotide sequences of the NBS-LRR-like *RGH*s. Interfamily BLASTn comparisons between members of *RGH* families 1, 2, and 3 revealed no significant similarity. However, the five members within the *RGH1* family are at least 60–98% similar and the two members within the *RGH2* family are 97% similar. Pairwise BLASTp comparisons revealed that members within a family contain 60–98% amino acid similarity, whereas pairwise interfamily comparisons among members of *RGH* families 1, 2, and 3 revealed 33% amino acid similarity or less. Pairwise intrafamily GCG-GAP comparisons revealed that members within a family were up to 87% similar at both the nucleic acid and amino acid level. Pairwise interfamily GCG-GAP similarity among members of *RGH* families 1, 2, and 3 was 47% or less at the nucleic acid level and 44% or less at the amino acid level. To the same extent, we did not observe interfamily cross-hybridization among the members of *RGH1*, *RGH2*, and *RGH3* under high-stringency wash conditions (0.1% SDS, 0.1× SSPE at 65°).

Genetic mapping and physical organization of the *RGH* families: Sequences corresponding to the *RGH*s from 80H14 were mapped back onto our high-resolution population. Allele-specific PCR primers and/or polymorphic-hybridization probes were developed for *RGH1a*, *1b*, *1d*, *1e*, and *3a* (Table 7). We were unable to obtain allele-specific primers for *RGH1c* and *2a* due to the monomorphic feature of the respective products amplified from the C.I. 16151 and C.I. 16155 mapping parents. Intragenic DNA sequence similarities between the C.I. 16151- and C.I. 16155-derived amplified *RGH* fragments are >95%, whereas the intragenic DNA sequence similarities between C.I. 16151- or C.I. 16155-derived *RGH* amplicons and Morex are ~80%. However, each of the allele-specific *RGH*s was genetically positioned in the physical interval that cosegregated with *Mla6*, *Mla14*, *Mla13*, and *Ml-Ru3*. This demonstrates that most, if not all, Morex-derived *RGH*s are also present in lines that contain characterized *Mla* specificities and that these *RGH* copies map to syntenic positions.

We also used sequences representing these *RGH*s as hybridization probes on BAC DNA fingerprinting filters (Figure 7) to identify additional *RGH* members on the BAC contig and derive a model for the physical organization of the *RGH*s associated with the *Mla* cluster. Indeed, another three additional *RGH*s, each belonging to one of the three families, were discovered on the adjacent BACs proximal to 80H14. By combining these hybridization results with data from the low-pass and BAC-end sequencing, at least 11 *RGH*s were found in this region.

TABLE 6

PCR primers derived from Morex BAC-end sequences and amplification on parental barley lines

BAC clones	Primers for R1 end (5′-3′)	Fragment size (bp)	Annealing temp.	Sequence origin[a]	PCR amplification on mapping parents[b]		Primers for T7 end (5′-3′)	Fragment size (bp)	Sequence origin[a]	Annealing temp.	PCR amplification on mapping parents[b]	
					CI 16151	CI 16155					C.I. 16151	C.I. 16155
80 H14	TGCTTTACCTCAAG TTGGCTGC	212	54	C.I. 15773	+	+	GGTTACATTAGAG CACTTGCACCC	422	C.I. 15773	54	+	+
	CGAAGGTGTGTGA TTTCGATGC						CCAAGGACGAGGA AATCAGTAGG					
	GGTGTGTGATTTC GATGCC	146	54	C.I. 16151	+	−						
	CAGGAGCCTGCAC CGTCT											
	GGTGTGTGATTTC GATGCC	189	54	C.I. 16155	−	+						
	TGCCAACTGTGTC GACGT											
401 K4	CAGAAGTGTTCAC TCATCCCCG	192	51	C.I. 15773	+	+	NA	NA	NA	NA	NA	NA
	AGATATAGCTTTC CATCGCACAATG											
711 N16 and 709 G18[c]	GGAATGCAATGTA AGGCTTAAACAC	310	53	C.I. 15773	+	+	NA	NA	NA	NA	NA	NA
	GCAATCACCAGGG TACTGATATGC											
721 K19	TTCAATAATCCCCT CAGTCGTAGC	255	56	C.I. 15773	+	−	GGTAGTAATCAAG CCAATTCCGC	457	C.I. 15773	53	+	+
	AACGGTTGCCAC CATCACTG						GCAATCACCAGGG TACTGATATGC					
257 G8	AATTCGTGAAGGG TTTCGTGG	447	56	C.I. 15773	+	+	TTCAATAATCCCCT CAGTCGTAGC	255	C.I. 15773	56	+	−
	GGATATTCCTCA GATCGGACAAGC						AACGGTTGCCAC CATCACTG					
175 D16	CTGACCAAAGTGA TCCTTAGCTCAG	327	53	C.I. 15773	+	+	CACCAAAAACTTT TACCCTC	445	C.I. 15773	50	+	+
	CGATAGCCATTGT GGAGTTGGAG						ATACCGTGACCTC TCTGCTC					
							TGTTTAATAATTT CAACACAAAG	272	C.I. 16151	51	+	−
							ATACCGTGACCTC TCTGCTC					

(*continued*)

TABLE 6

(Continued)

BAC clones	Primers for R1 end (5′-3′)	Fragment size (bp)	Annealing temp.	Sequence origin[a]	PCR amplification on mapping parents[b]		Primers for T7 end (5′-3′)	Fragment size (bp)	Sequence origin[a]	Annealing temp.	PCR amplification on mapping parents[b]	
					CI 16151	CI 16155					CI 16151	CI 16155
540 G23 and 555 A13 and 559 A6[c]	CCATTTCAACAATC CAGTGTGCTC ACGCAAAAAACGT GGGTGC	386	70	C.I. 15773	−	−	CCGATGAGGGAAG CAATCTGAC AGAGCAAAAGCAG CAAAAGGC	329	C.I. 15773	56	+	+
206 D11 and 206 120[c]	NA	NA	NA	NA	NA	NA	CTGGTTTGTTGTT GCTATGCGTTG TCATTTGGTGTGG GGCAAAG	470	C.I. 15773	56	+	+
							TTTTGATCTGATC CGGCGC TCATTTGGTGTGG GGCAAAG	298	C.I. 16155	56	−	+

NA, data not available.

[a] Accessions C.I. 16151 and C.I. 16155 contain the *Mla6* and *Mla13* specificities, respectively. C.I. 15773 is the designation for Morex.

[b] +, amplification; −, lack of amplification.

[c] BACs of identical size and inserts.

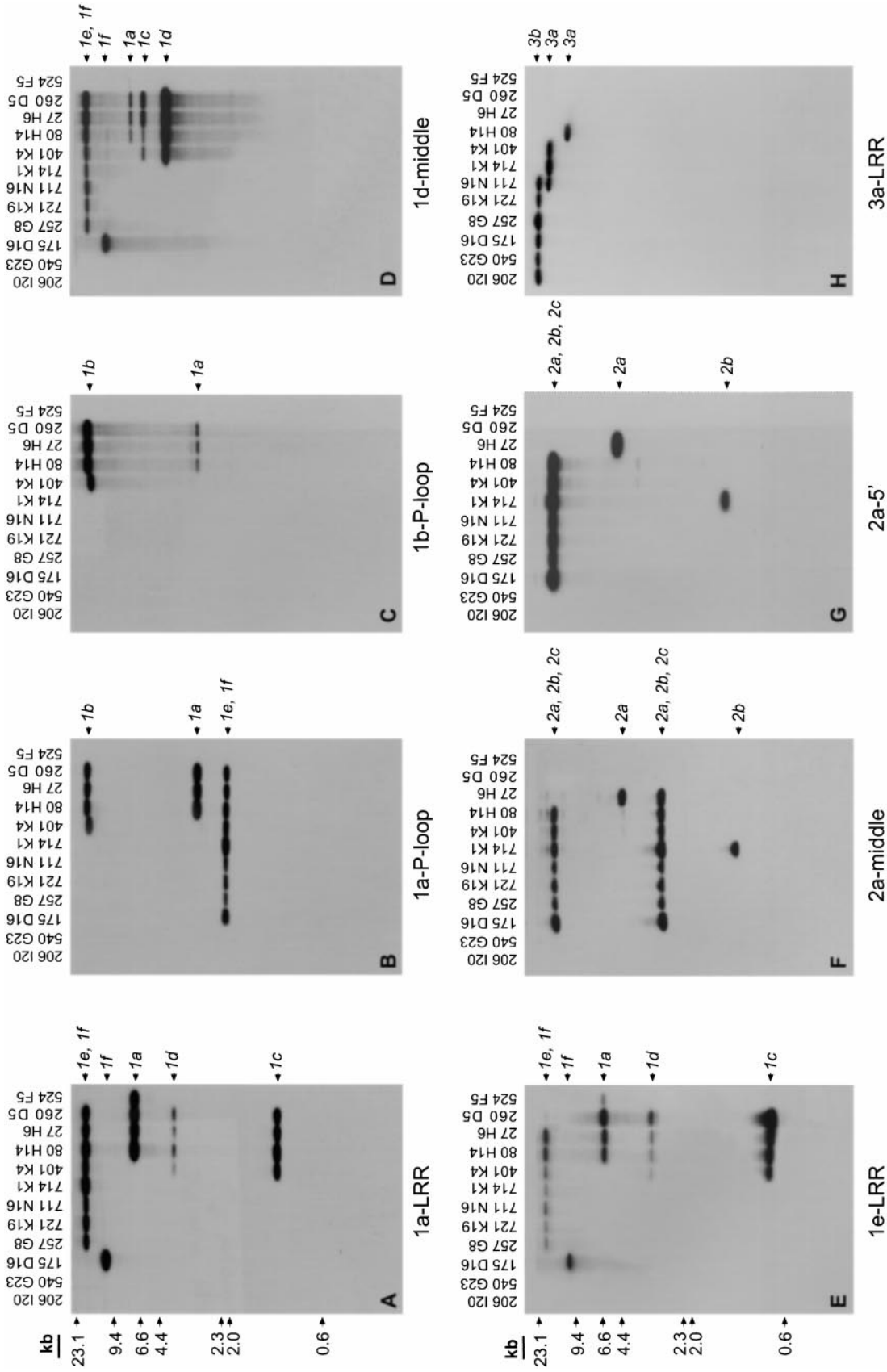
TABLE 7

***RGH*-specific primer pairs**

Primer designation	Primer sequences (5′ → 3′)	Fragment size (bp)	*RGH* designation	Region of *RGH* ORF	Annealing temperature
39F236	TCCAATTCCGCAAGCTCTGAG	571	*RGH1a*	P-loop	56
39B318	ACCTCTCCTACAACATGGAGGATG				
39F146	AGCAGATACCTCGCCTAGACCATC	334	*RGH1a*	Middle	56
39B184	GGTAAAGCCAAAGAGTGAATGGG				
39F13	GGTTACCATCCTCTTTCGTCACC	582	*RGH1a*	LRR	56
39B95	GGAGGCTCGTTGTGTCTCTGAATAC				
15B02F1	TTTCCCAACCCACCAAATCC	307	*RGH1b*	P-loop	58
15B02B9	TCGGCGTGTTCAAGAAAGGC				
15G06F34	CGAGCAAAAACAGGCTAGTCCC	527	*RGH1d*	Middle	56
15G06B21	CAACAGGAACAATTTAGGCAGTCG				
38F13	GCTTGAGATCATGCACACTGTCC	467	*RGH1e*	P-loop	52
38B58	TGTTAGAGGCATCGTCTTCGGTC				
38F19	TGGTTCCAACTGGTGTGTTGC	426	*RGH1e*	LRR	54
38B27	CCCCAATGATTTCCACGTCC				
15A08F2	AACATGGAGATGATGGAGGCG	750	*RGH2a*	5′	60
15A08B3	TTGCGAGAGTATGCTGGTGAGG				
FWA57F	ACCCTCGCCAGACAAGTTTACC	165	*RGH2a*	P-loop	56
FWA55B	GAAAAGCAGCTTATGCACGTCG				
38IF2	CAGACATGCTGTGCAGATACCTCC	420	*RGH2a*	Middle	54
38IB4	TTCGGCTATCCACTGCTTCACC				
FWA61F	CCTTTTCTGGATTGCTCAAGTGC	277	*RGH2a*	LRR	56
FWA59B	ATCTGGGTTGGAAGCTCCACACTC				
FWA62F	CTTTGCACTCTGGGCATAATTGAC	341	*RGH2a*	LRR	56
FWA63B	AAGCTGGACAAGATGAAGCAGC				
FWA53F	TCGGCATCCTTATGATCCAGC	202	*RGH3a*	P-loop	56
FWA51B	TGCTCGACATCGAACCAAACC				
80H14R1F3	CGTGATTCCCAAGGAAATTGC	500	*RGH3a*	Middle	56
80H14R1B6	GTTGCCATGTAGCTTGAGTGAGC				
80H14R1F30	TGCTTTACCTCAAGTTGGCTGC	212	*RGH3a*	LRR	56
80H14R1B35	CGAAGGTGTGTGATTTCGATGC				

These new *RGH*s fall into the three previously described families, which brings the total to 6 members in the *RGH1* family, 3 members in the *RGH2* family, and 2 members in the *RGH3* family. Presently, 7 of these NBS-LRR-like *RGH*s cannot be separated from the *Mla* locus by recombination events. Due to the large duplication proximal to 80H14, we were unable to develop distinct polymorphic markers between *721K19-R1.1* and *175D16-T7* (Figure 5). Therefore, at this time, we cannot determine whether the proximal 4 *RGH*s are genetically within the *Mla* cosegregating interval. Figure 8 illustrates a model for the physical organization of the *RGH*s

Figure 7.—DNA gel blot hybridization of *RGH* probes onto *Eco*RI-digested Morex BACs. All membranes were washed at high stringency (0.1× SSPE, 0.1% SDS for 30 min at 65°). Probe DNAs were amplified from the primer pairs listed in Table 7. (A–H) Probes representing *RGH* domains are shown at the bottom; at the top are the BAC clone designations. At the left of A and E are λ-*Hin*dIII DNA size standards. *RGH* member designations are indicated on the right side of A–H. (A) Probe 1a-LRR was amplified from primer pair 39F13 and 39B95 and corresponds to the LRR region of *RGH1a.* Four highly similar *RGH1* members hybridized to this probe. (B) Probe 1a-P-loop was amplified from primer pair 39F236 and 39B318 and corresponds to the P-loop region of *RGH1a.* Four highly similar *RGH1* members also hybridized to this probe. (C) Probe 1b-P-loop was amplified from primer pair 15B02F1 and 15B02B9 and corresponds to the P-loop region of *RGH1b.* The hybridization pattern revealed two highly similar members of the *RGH1* family. (D) Probe 1d-middle was amplified from primer pair 15G06F34 and 15G06B21 and corresponds to the LRR region of *RGH1d.* The hybridization pattern indicates that there are five highly similar members of the *RGH1* family. (E) Probe 1e-LRR was generated from primer pair 38F19 and 38B27 and corresponds to the LRR region of *RGH1e.* Four copies of the *RGH1* family hybridized to this probe. (F) Probe 2a-middle was amplified from primer pair 38IF2 and 38IB4 and corresponds to the region between the P-loop and LRR of *RGH2a.* The hybridization result showed the existence of three highly similar members of the *RGH2* family. (G) Probe 2a-5′ was amplified from primer pair 15A08F2 and 15A08B3 and corresponds to the 5′ end of the P-loop region of *RGH2a.* Three highly similar copies hybridized to this probe. (H) Probe 3a-LRR was generated from primer pair 80H14R1F30 and 80H14R135 and corresponds to the LRR region of *RGH2b.* The hybridization pattern indicated that there are two copies of this region.


kb
23.1
9.4
6.6
4.4
2.3
2.0
0.6
206 I20
540 G23
175 D16
257 G8
721 K19
711 N16
714 K1
401 K4
80 H14
27 H6
260 D5
524 F5
1e, 1f
1f
1a
1d
1c
1b
2a, 2b, 2c
2a
2b
3b
3a
A
1a-LRR
B
1a-P-loop
C
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1d-middle
E
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F
2a-middle
G
2a-5'
H
3a-LRR

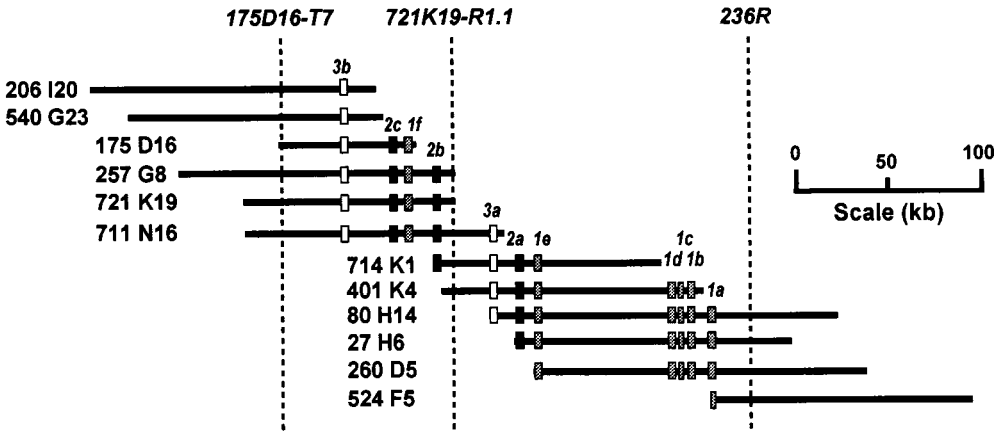

Figure 8.—Physical model illustrating the minimum-tiling path of NBS-LRR resistance-gene homologues on BACs in the cosegregating *Mla* interval. This model was derived by hybridizing probes derived from the BAC-end primers listed in Table 6, the *RGH* primers listed in Table 7, and RFLP markers shown in Figure 5, to DNA-gel blots containing the *Eco*RI-digested BACs shown in Figure 7. There are six distinct copies of the *RGH1* family dispersed over ~150 kb, three copies of the *RGH2* family covering ~100 kb, and two copies of the *RGH3* family also covering ~100 kb. The two markers, *236R* and *721K19-R1.1*, define the current *Mla* cosegregating interval (Figure 5). However, the segment between *236R* and *175D16-T7* contains all of the known *RGH*s and therefore defines the physical limit of *Mla*-associated NBS-LRR gene families to 240 kb.

associated with the *Mla* cluster. The segment between *236R* and *175D16-T7* contains all of the known *RGH*s, and therefore defines the physical limit of *Mla*-associated NBS-LRR gene families to 240 kb.

The use of the fingerprinting restriction endonuclease *Eco*RI simplified the interpretation of the physical organization of the *RGH* family members. During initial library construction, BAC inserts were ligated into the *Hin*dIII cloning site of pBeloBAC11. Restriction digestion of these BACs with the enzyme *Eco*RI releases asymmetric BAC-end fragments. Hence, migration of an *Eco*RI BAC-end fragment will be distinguishable from a BAC-internal fragment. This was advantageous because, due to the duplicated segments in the *Mla* region, additional members of a gene family could be exposed upon hybridization with various *RGH* domain probes. For example, in Figure 7, A and D, a *RGH1e*-comigrating fragment corresponding to *RGH1f* in BACs 711N16, 721K19, and 257G8 is revealed by the altered migration of the BAC end from 175D16. In F and G, the second member of the *RGH2* family is revealed by BAC 714K1-end sequencing and is also shown by the altered migration of the end via the *Eco*RI digest of this BAC. Furthermore, a third *RGH2* member is shown by hybridization of the non-714K1 overlapping BAC, 175D16. These additional copies were indistinguishable when DNA gel blots of *Hin*dIII-restricted BACs were probed with the *RGH* probes in Figure 7.

Suppressed recombination within the *Mla* cluster: Generally, the ratio of physical to genetic distance is low in regions near the centromere and high in regions toward the telomere (Schnable *et al.* 1998). The average relationship between genetic and physical distance in barley, based on a genome size of 5300 Mb and a genetic map of 1250–1453 cM, is 4.2–3.7 Mb/cM (Graner *et al.* 1991; Kleinhofs *et al.* 1993). On the basis of cytogenetic analysis, Pedersen and Linde-Laursen (1995) and Sorokin *et al.* (1994) reported a ratio of 1.0 and 2.0 Mb/cM, respectively, in the short arm region of barley chromosome 5 (1H).

As shown in Table 8, we compared physical to genetic distance ratios in eight intervals cosegregating with and adjacent to the *Mla* cluster. It appears that intervals closer to the *Mla* cluster undergo less recombination, at least in progeny of the C.I. 161561 × C.I. 16155 mapping cross. We observed no recombinants in the *236R* to *721K19-R1.1* interval, which contains nearly all the NBS-LRR resistance-gene homologues. This lack of recombination delimits the physical to genetic distance ratio to 5 Mb/cM. However, regions immediately flanking the *Mla* cluster appeared to recombine at a higher rate than the average for the barley genome and the short arm of chromosome 5 (1H).

DISCUSSION

To determine the molecular processes that mediate host resistance, our aim is to isolate a number of resistance specificities of the *Mla* locus. In this article, we describe the identification of several tightly linked DNA markers and the establishment of a *Mla*-spanning YAC and BAC contig. This *Mla*-spanning contig has facilitated the discovery of 11 NBS-LRR resistance-gene homologues, at least 7 of which cosegregate with the *Mla* locus.

Highly dissimilar NBS-LRR resistance-gene-like families are physically associated with the *Mla* cluster: In the past several years, major long-term efforts have reached fruition in the cloning of resistance genes in a variety of plant species (reviewed by Michelmore and Meyers 1998; Ronald 1998). Although the isolated genes confer resistance to a diverse range of pathogens, those involved in gene-for-gene interactions between host and pathogen share various conserved motifs. These include a serine-threonine protein-kinase domain, a leucine zipper (LZ), a Toll and interleukin-like receptor domain

TABLE 8

Ratios of physical to genetic distance in eight intervals spanning the *Mla* cluster

Interval	Physical distance	No. of recombinants	kb/crossover	kb/cM[a]
RGH:b6-236 → Fr1062	~250 kb	7	36/1	1262
Fr1062 → FW108	~25 kb	5	5/1	176
FW108 → 234L	<30 kb	2	15/1	<530
234L → mwg2197	~60 kb	1	60/1	2120
mwg2197 → 236R (Mla)	~120 kb	2	60/1	2120
236R → 721K19-R1.1	~140 kb	0	140/0	<4943
721K19-R1.1 → 175D16-T7	~80 kb	9	9/1	353
175D16-T7 → 206I20-T7	~85 kb	5	17/1	706

[a] It was previously determined that the 8.1-cM *Hor1 → Hor2* interval on chromosome 5S had 286 crossovers in our high resolution mapping population (DeScenzo *et al.* 1994). This is equivalent to 0.0283 cM/crossover. The kb/cM ratio is based on the average resolution per recombination event in the *Hor1 → Hor2* interval in the C.I. 16151 × C.I. 16155 cross.

(TIR), NBS, and LRRs. The most prevalent class of cloned plant-resistance genes contains the nucleotide-binding site combined with various lengths of a leucine-rich repeat. This NBS-LRR class is predicted to encode intracellular proteins (Michelmore and Meyers 1998; Ronald 1998). The 11 *RGH*s that are physically present on the Morex BAC contig belong to three distinct families of the NBS-LRR class of resistance genes. Of these *RGH*s, 7 have been genetically delineated to the region that contains the *Mla6*, *Mla14*, *Mla13*, and *Ml-Ru3* specificities. Previous reports have shown that NBS-LRR genes can be physically juxtaposed to genes defining additional components of the resistance response. *Prf*, a NBS-LRR gene, is located adjacent to *Pto*, encoding a serine-threonine kinase, both of which define essential components of race-specific resistance to bacterial speck disease in tomato (Salmeron *et al.* 1996). Among >150 kb of DNA sequence surveyed, no sequences exhibiting similarities to kinases were identified in the contig spanning *Mla*. Thus, the present data suggest that the *Mla* locus contains only NBS-LRR-type *RGH*s.

The majority of plant resistance genes appear to be organized as complex clusters. For example, the *Xa21* resistance gene family of rice and the *Cf-2* family of tomato are assembled as single, locally restricted clusters of homologous genes (Song *et al.* 1995, 1997; Dixon *et al.* 1996, 1998). The *Dm3* locus of lettuce and the *Cf4*/*Cf9* locus of tomato define two examples in which numerous related copies of resistance gene homologues are spread over several megabases within one chromosome (Anderson *et al.* 1996; Parniske *et al.* 1997; Meyers *et al.* 1998a,b; Shen *et al.* 1998). Finally, the related *L* and *M* genes of flax are located on different chromosomes (Lawrence *et al.* 1995; Anderson *et al.* 1997). In contrast, we have observed at *Mla* an interspersed arrangement of three unrelated NBS-LRR-like gene families (Figure 8). Additionally, these three *Mla*-cosegregating *RGH* families do not have significant similarity to the barley *Hv-b6 RGH* family, positioned 0.48–0.62 cM distal to the *Mla6*, *Mla13*, *Mla14*, and *Ml-Ru3* specificities (Figure 5). This multifamily organization of resistance genes and resistance-gene homologues is comparable to the recent report of mixed clusters of NBS-LRR *RGH*s of rice, each harboring at least two highly dissimilar NBS-LRR genes (Leister *et al.* 1999).

***RGH* families and *Mla* resistance specificities:** The physical organization of the NBS-LRR-like sequences associated with the *Mla* locus was obtained from cultivar Morex, a Manchuria-type barley (Kleinhofs *et al.* 1993). The cultivar Manchuria does not have any known *Mla* specificity and Morex also does not confer resistance to our isolates used for mapping the *Mla6*, *Mla13*, *Mla14*, and *Ml-Ru3* specificities. However, the cosegregating feature of the three *RGH* families within the genetically delimited (*Mla6*, *Mla14*, *Mla13*, and *Ml-Ru3*) interval indicates that they may be homologues of individual *Mla* resistance specificities. Indeed, it has been shown that susceptible cultivars or subspecies do harbor homologues of resistance genes at syntenic positions. The *Cf0* locus in susceptible *Lycopersicon esculentum* contains a homologue of the *Cf9* resistance gene that was introgressed from *L. pimpinellifolium* (Parniske *et al.* 1997). Likewise, a homologue of the *Xa1* resistance gene in the resistant cultivar IR-BB1 is present at the same locus in the susceptible near-isogenic line IR24 (Yoshimura *et al.* 1998). Because *Mla6* and *Mla14* were introgressed from the wild barley *H. spontaneum* nigr. (reviewed by Jørgensen 1994), it is conceivable that the *RGH* families at *Mla*, derived from the susceptible cultivar Morex, represent homologues of individual *Mla* resistance specificities.

There are, however, also cases in which susceptible lines lack homologues of resistance genes. For example, the *Xa21* bacterial-blight resistance locus that was introgressed from wild rice, *Oryza longistaminata*, does not exist in cultivated rice, *O. sativa* (Song *et al.* 1995, 1997).

Similarly, *RPM1* in Arabidopsis is present in ecotype Columbia but absent in at least six other naturally occurring accessions (Grant *et al.* 1995). This does not appear to be the case for the *Mla* cluster. As described above, we have been able to amplify homologous sequences corresponding to several of the *RGH*s from C.I. 16151 (containing *Mla6* + *Mla14*) and C.I. 16155 (containing *Mla13* + *Ml-Ru3*) with the Morex-derived *RGH* primers described in Table 6. Additionally, these homologs genetically cosegregate with the *Mla6*, *Mla13*, *Mla14*, and *Ml-Ru3* specificities in our high-resolution mapping population. Taken together, these data provide the possibility that the Morex-derived *RGH* families represent homologues of single *Mla* resistance specificities.

Mutational studies uncovered two genes, *Rar1* and *Rar2*, required for *Mla*-specified resistance responses (Torp and Jørgensen 1986; Jørgensen 1988, 1996; Freialdenhoven *et al.* 1994). *Rar1*, located on barley chromosome 2, has been recently isolated and encodes a novel protein that is likely to function in disease resistance signaling (Lahaye *et al.* 1998; Shirasu *et al.* 1999). *Rar1* and *Rar2* are required for the function of some but not all tested *Mla* specificities (Jørgensen 1988, 1996). Our finding of three unrelated *RGH* families at *Mla* could provide a simple explanation for the differential *Rar*-gene requirements if some of the *Mla* specificities are encoded by one *RGH* family and another set are encoded by a different *RGH* family. In this scenario, distinct NBS-LRR families would have the capacity to activate downstream signaling components. The availability of altered-specificity mutants for *Mla1* (S. Somerville, unpublished results), *Mla6* (R. P. Wise, unpublished results), and *Mla12* (Torp and Jørgensen 1986), each exhibiting differential requirements for *Rar1* and *Rar2*, is expected to facilitate the identification of individual *Mla* resistance specificities and to provide a molecular basis to test our hypotheses.

Recombination is suppressed in highly polymorphic regions of the genome: The relationship between physical and genetic distance varies throughout the eukaryotic genome. This variation depends on many factors, including the composition of surrounding DNA sequences (Gustafson *et al.* 1990; Leitch *et al.* 1991; Schwarzacher and Heslop-Harrison 1991; Werner *et al.* 1992; Gill *et al.* 1993; Jiang and Gill 1993; Kota *et al.* 1993; Leitch and Heslop-Harrison 1993; Schmidt *et al.* 1994; Pedersen and Linde-Laursen 1995). As shown in Table 8, the ratio of physical to genetic distance varies >10-fold in intervals adjacent to and cosegregating with the *Mla* cluster. Indeed, recombination was not observed in the cosegregating physical interval that encompasses the *Mla6*, *Mla14*, *Mla13*, and *Ml-Ru3* specificities and the three associated *RGH* families. This observation could be due to lack of pairing and subsequent strand exchange between homologous regions in the C.I. 16151 and C.I. 16155 parents of our mapping cross. These two accessions were originally chosen because of their high rate of hordein-polypeptide polymorphism and easily detectable differences in infection type (Mahadevappa *et al.* 1994). However, it may be that suppression of recombination occurs within the *Mla* cluster because of this high rate of polymorphism. This recombination suppression contrasts with observations at the *Rp1* rust-resistance cluster in maize (Collins *et al.* 1999), where high rates of recombination and unequal crossover have been shown to be a source of new resistance specificities (Richter *et al.* 1995).

The *Mla6* allele was originally introgressed into cultivated barley from *H. spontaneum*, a wild relative of *H. vulgare* (Jørgensen 1994). Suppressed recombination has been observed in other introgressed regions associated with disease resistance, such as the *Mi* (van Daelen *et al.* 1993) and *Tm2-a* (Ganal *et al.* 1989) loci in tomato. There is a distinct difference in these two cases, however, as the *Mi* and *Tm2-a* loci are physically close to the centromere where regions of heterochromatin were postulated to suppress recombination in this area of the chromosome.

In summary, we have established a detailed physical map of the *Mla*-spanning region and presented the physical organization of different members of *R*-gene homologues within the contig. New *Mla* mutants should allow us to determine the location of different members of this resistance-gene family and ultimately define specific regions of the gene (and therefore, protein) that are important in host-pathogen recognition. Determination of the sequence differences among mutant alleles will provide important clues in our long-range goal to understand the evolution and molecular mechanisms of host-pathogen interaction among members of the Gramineae and obligate biotrophs.

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