Genome mapping in plants

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Genome mapping permits the study of morphological, physiological, and developmental processes in which genetic variants exist, and requires minimal *a priori* information. Further exploitation of the polymerase chain reaction, yeast artificial chromosomes, and comparative analysis of distantly related taxa, will contribute greatly to the fundamental understanding of plant biology and crop production.

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Introduction

Genome mapping, a synthesis of concepts from classical genetics using tools from molecular biology and methods from biometry, is a powerful approach for the study of plant biology. Genome mapping is rooted in classical genetic linkage analysis, but recent technological advances facilitate the mapping of genes responsible for either simple or complex phenotypes. Plants are well suited to genetic mapping because of their short generation times, the ease with which large populations can be grown, and their amenability to artificial crosses. Extensive collections of variants in plant morphology, physiology, and development are available that provide fertile sources of genes for study.

Map-based cloning (that is, the cloning of genes underlying discernible phenotypes based upon map position) facilitates the isolation of genes affecting processes of developmental and/or economic importance, with minimal *a priori* information. Map-based cloning in higher plants is complicated by physically large genomes, prominent repetitive DNA fractions, and polyploidy. Consequently, many developmentally important genes are being cloned in plants such as *Arabidopsis*, by insertional mutagenesis [1] or subtractive hybridization [2]. Crop plants, however, are cultivated for attributes that are not found in model systems, such as the cotton fiber, maize 'ear', or tomato 'fruit' (berry). The cloning of genes associated with such attributes, which are the basis of agricultural productivity, will require the development of high-density restriction fragment length polymorphism (RFLP) maps and yeast artificial chromosome (YAC) libraries for major crops and their close relatives.

Genetic mapping

Plant genetic maps

Detailed genetic maps, based largely upon RFLPs, have been constructed for many plants [3]. The most outstanding among these are the maps of tomato (1400 markers distributed over 12 chromosomes) [4••], and *Arabidopsis* (500 markers distributed over five chromosomes) [5–7]. Recent additions to the growing list of plant genetic maps include conifer [8], loblolly pine [9], barley [10], peanut [11], *Brassica rapa* [12], rye [13], wheat [14], *Cuphea* [15], sugarcane (B Sobral, personal communication) and cotton (AH Paterson, unpublished data).

The integrity of a genetic map can be assessed statistically [16], but biological tests have been reported recently. Mapping of length polymorphisms in subtelomeric tandem arrays of a 162 base pair element showed that the genetic maps of four tomato chromosomes are within 5–10 cM of the physical chromosome ends [17]. This is in contrast to recent results from *in situ* hybridization of mapped low copy number clones to rice chromosomes, suggesting that the genetic map falls short of the physical chromosome ends [18]. This conflict has yet to be clearly resolved.

Most primary genetic maps of plants have been made in pedigrees chosen for their high DNA marker allele diversity. While many of these crosses harbor agriculturally useful genes, they are unlikely to produce genotypes suited to the needs of the producer, or to the tastes of the consumer. The advantage of using wide crosses for genetic mapping is a consequence of the fact that the elite gene pools of many important crops are genetically narrow [19]. This indicates that there is an immediate, practical need for DNA

Abbreviations

PCR—polymerase chain reaction; **QTL**—quantitative trait locus; **RAPD**—randomly amplified polymorphic DNA;
RFLP—restriction fragment length polymorphism; **STM**—sequence-tagged microsatellite; **YAC**—yeast artificial chromosome.

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markers that facilitate the genetic analysis of closely related individuals. Solutions to the problem of minimal genetic variation are available, using hypervariable DNA markers associated with arrays of short tandemly repeated sequences [20]. Recent developments have streamlined identification of such markers, and maps of the human [21•], mouse [22••], and rat [23] genomes now include polymerase chain reaction (PCR) based sequence-tagged microsatellites (STMs). The construction of genomic DNA libraries enriched for STMs was described recently [24]. These allele-rich markers serve the needs of both genetic and physical mapping, and are present in many plants [25].

PCR-based genotyping promises to streamline genetic mapping [26]. A limitation of PCR-based genotyping is that the development of STMs requires a large investment in sequencing. Randomly amplified polymorphic DNA (RAPD) PCR [27] and arbitrary primer PCR [28] minimize this cost by using primers of quasi-arbitrary sequence for DNA amplification. One genetic linkage map based largely on RAPDs has been published [7], and another based upon arbitrary primer PCR is underway (B Sobral, personal communication). Dominant inheritance of most RAPD markers sacrifices much information in F2 populations [29]. Further, the reliability of many RAPD markers may not be sufficient for the stringent requirements of linkage mapping, although arbitrary primer PCR markers appear to be more reliable. While the limitations of RAPDs are partially alleviated in homozygous [7] or hemizygous [30] populations, we believe that RAPDs will only find limited use in the construction of primary genetic maps. However, we emphasize that PCR using arbitrary primers is valuable for enriching predefined genomic regions for DNA markers.

Comparative mapping of plants

Many DNA sequences that encode proteins are conserved across a wide range of organisms, while noncoding sequences are more freely divergent. Genetic mapping of conserved DNA sequences (such as cDNAs) permits the investigation of the order of genes along chromosomes in distantly related taxa. Through comparative mapping, parallelism in gene order (synteny) along the chromosomes, as well as breakpoints responsible for differences in gene order, have been demonstrated for tomato and potato [4••,31,32], and sorghum and maize [33]. Comparative mapping suggests that remarkably few macroevolutionary events may distinguish plant species. Comparative maps have practical applications too: by extrapolating map information from one taxon to another, efficient high-density mapping of related taxa can be done simultaneously [3,31,32].

Like cDNAs, some sequence tagged sites are conserved across taxa, permitting PCR to contribute to comparative mapping [34••]. Successful PCR amplification of 30 million year old specimens [35••] adds another dimension to comparative mapping – a vertical picture through time, as well as a horizontal picture across extant taxa. Comparative mapping may help to unravel the genetic complexities of polyploids. Wheat, soybean, cotton, and other important crops are polyploid, harboring two or more genomes that are distantly related but do not normally pair during meiosis. In the cotton RFLP map (AH Paterson, unpublished data), about 10% of RFLP markers reveal polymorphism at corresponding loci on each of the two genomes. These markers provide a framework for establishing relationships between the two genomes. As these relationships become clear, each DNA probe mapped to one genome will also, in effect, be mapped to the other. The development of high-density maps for cotton and other polyploids will be accelerated by delineating relationships between homeologous chromosomes.

Phenotype mapping and gene tagging

Phenotype mapping is the primary justification for constructing genetic maps in plants. Closely linked DNA markers facilitate the indirect selection of traits that are difficult to measure, and provide a starting point for map-based cloning of genes of developmental and/or economic importance. DNA markers linked closely to simply inherited traits can be isolated efficiently using RAPD PCR on near-isogenic lines [36••]. Recently, this powerful technique has been extended to the enrichment of any genomic region for DNA markers, using synthetic DNA pools [37•,38•]. New markers have been isolated that are linked to the tomato developmental mutations *jointless* and *never-ripe* [38•], and lettuce downy mildew resistance loci [37•].

The DNA pooling approach raises the possibility of isolating markers for quantitative trait loci (QTLs) [37•]. This is especially appealing, as QTL mapping requires large populations to discern small phenotypic effects of numerous unlinked loci. Both theoretical expectations and *post hoc* analysis of previous QTL mapping results, suggest that only QTLs with unusually large effects can be detected using DNA pools (G Wang and AH Paterson, unpublished data). However, once QTLs have been mapped by current methods [39,40•,41], synthetic DNA pools are an excellent means of enriching the genomic region of a QTL for DNA markers. Such markers might be valuable for high-resolution mapping of the QTL [42] or introgression of the QTL into different genotypes.

Physical mapping and map-based cloning

Pulsed-field electrophoresis [43] and YAC DNA vectors [44] permit large regions of plant genomes to be dissected and cloned. These tools allow genes to be isolated based solely on their genetic map position.

Megabase DNA preparation

A reliable method for the isolation of relatively intact, unsheared plant DNA is a prerequisite for pulsed-field

electrophoresis and YAC cloning. Megabase DNA isolation procedures are available for many plants, the most recent additions being soybean [45], rice [46], wheat, barley and rye [47]. The recurring method is to embed plant protoplasts in agarose plugs, followed by lysis, proteinase digestion, and washing. Recently, we developed a procedure, adapted from [48], for embedding plant protoplasts in agarose microbeads, which offers significant advantages over plugs (RA Wing, S-S Woo, VK Rastogi, H-B Zhang, AH Paterson, SD Tanksley, unpublished data).

Genetic versus physical distance

Once molecular markers have been shown to be genetically linked to a target gene, it is prudent to establish the relationship between genetic and physical distance in the region surrounding the gene. This information will help to determine a chromosome walk or jump. The average relationship between the genetic and physical distance of a genome is readily calculated from the genome size and length of the genetic map. However, actual values for any specific location vary widely from the average. In the tomato, 1 cM averages 900 kb, but in the *Tm2a* region of chromosome 9, 1 cM is 4–16 Mb [49]. This physical distance is currently well beyond the capabilities of map-based cloning. In wheat, where the kb : cM ratio is much larger than in tomato, 1 cM is approximately equal to 1 Mb in the region of the α-amylase gene on chromosome 6 [47].

Yeast artificial chromosome libraries

Since the demonstration that large human DNA fragments can be maintained in YACs [44] many groups have set out to make plant YAC libraries. YAC libraries have been constructed for *Arabidopsis* [50,51], tomato [52], maize [53] and rice (Y Umehara *et al.* Abstract #141, Plant Genome I, San Diego, November 9–11, 1992). In tomato, Martin *et al.* [52] obtained 22 000 clones and screened half of the library with RFLP markers tightly linked to two disease resistance loci, *Tm2a* and *Pto*. Five YACs were isolated in this screen, which mark starting points for chromosome walks to these genes. Recently, a maize YAC library with three haploid genome equivalents was constructed [53]. The library was assembled in such a way that it can be rapidly screened by PCR [54]. Richards *et al.* [55] recently constructed a half-YAC library and isolated two biologically functional *Arabidopsis thaliana* telomeres in yeast.

Complete physical maps

Physical maps are valuable in studying the organization and evolution of plant chromosomes. *In situ* hybridization provides a 'global' view of genome organization [56]. Contig maps, assembled using high-density RFLP maps, cosmids and YACs, provide a 'local' view of the genome. An overlapping cosmid map comprising 90–95% of the *Arabidopsis* genome has been constructed [57]. Recently Hwang *et al.* [58] described preliminary data to assemble a set of overlapping YACs for the entire *Arabidopsis* genome. The authors isolated 125 YACs using RFLPs across the five linkage groups. These YACs represent 30% of the *Arabidopsis* genome. Ultimately, the authors plan to assemble a blot comprising the minimum number of YACs to overlap the entire genome, to aid in genetic mapping and chromosome walking.

Gene identification

The most direct approach to gene identification from a candidate YAC or cosmid is to complement a mutant by transformation. Giraudat *et al.* [59] recently demonstrated mutant complementation of the *abi3* gene using a subclone from one of three overlapping cosmids isolated using a linked RFLP. A YAC or a cosmid can be used as a probe to identify cDNAs specific to the candidate region. Mutant and normal cDNAs can then be sequenced to look for potential mutations. The best candidate cDNA can then be used for mutant complementation, or to create a mutation using antisense technology. Arondel *et al.* [60••] recently isolated a set of overlapping YACs which covered the *fad3* locus of *Arabidopsis*. One YAC was used to screen a cDNA library made from developing seeds from a closely related species (*Brassica napus*). A heterologous cDNA was isolated and shown to complement the *fad3* allele.

When no cDNA can be found, the YAC insert must be subcloned into overlapping fragments, and complementation attempted with each subclone independently. This is time-consuming and labor-intensive. Several groups have demonstrated stable integration [61–63] or complementation [64] of mammalian mutations with entire YACs. Transfer of whole YACs into plants will streamline gene identification.

Conclusion

For much of the 20th century, biologists have been aware of the potential that genetic markers hold for the investigation of complex questions in biology. The novel field of genome mapping now has a sufficient basis of technology and information to realize this potential in a few well-studied plants. Exploitation of comparative map information will promote continued technological improvement, and facilitate extension of genome mapping to less facile species. We believe that the next few years will provide tangible results of genome mapping progress in the form of cloned genes, improved cultivars, and a better understanding of plant biology.

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