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Models and meiosis in the 'omics era

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Abstract. The recent publication of draft genomic sequences, especially of model organisms, together with the build up and assembly of functional genomic resources, has enabled unprecedented access to the genes and genomes of hitherto intractable species, and to novel areas of research. To illustrate the importance and potential of this infrastructure and data stream, two projects are described. The first part details how we are coupling the genomic and proteomic resources of *Arabidopsis* to the genetic and cytological resources of rye in order to further our understanding of the genetic control of meiosis in grasses and cereals. The second part describes the current status of our project to develop and exploit for gene discovery the temperate grass *Brachypodium distachyon* as a genomic bridge between rice and the temperate grasses and cereals.

Key words: *Brachypodium distachyon*, genomics, meiosis, models, proteomics, rye

Introduction

The recent publication of draft genomic sequences of some model organisms, such as *Arabidopsis thaliana* (The Arabidopsis Genome Initiative 2000) and rice (GOFF et al. 2002; YU et al. 2002), have enabled unprecedented access to their genomes, genes and proteins. The development of these genomic and proteomic resources in models, the new 'omics era, is now being exploited to elucidate the function of genes and their products in related species. To illustrate the importance and potential of this powerful capacity for knowledge transfer between species, two projects are described. The first part details how we are coupling the genomic and proteomic resources of *Arabidopsis* to the genetic and cytological resources of rye in order to further our understanding of the genetic control of meiosis in grasses and cereals. The second part describes the current

status of our project to develop and exploit for gene discovery the temperate grass *Brachypodium distachyon* as a genomic bridge between rice and the temperate grasses and cereals.

Comparative genomic and proteomic dissection of meiosis in rye

Meiosis is a specialised form of nuclear division that halves the zygotic chromosome number and enables genetic recombination through crossing over and independent segregation of genes. Much of our knowledge of the genetic control of this process has come from studies of the cytology and molecular biology of model organisms, such as *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* (KLECKNER 1996; ROEDER 1997). However, despite considerable advances in our understanding of meiosis in recent years, fundamental questions still remain unanswered. For example, we have little notion as to how homologous chromosomes recognise one another and pair together. We are only just beginning to piece together the puzzle as to how recombinogenic genes and their products interrelate, and what governs the distribution of crossovers. We have little idea how homologous bivalent formation is controlled in polyploids, and we are not certain to what extent meiosis works in the same way in different organisms.

Although meiosis in yeast is particularly amenable to scientific investigation, there is no guarantee that the process works the same way in other organisms. Accordingly, we have adopted rye as a meiotic model, not only because it represents the agronomically important cereals and grasses and has wheat as its closest ally, but also because it has excellent biological attributes and a long history of experimental analysis. Rye provides bountiful supplies of easily staged pollen mother cells, which are well described by light and electron microscopy, and which constitute excellent substrates for molecular cytogenetic and molecular biological investigation. Also, a unique collection of meiotic mutants is available, which are maintained by selfing heterozygotes in families segregating for the mutant alleles (SOSNIKHINA et al. 2005). We have focussed upon three non-allelic mutants from this collection, each of which has a distinctive meiotic phenotype. Mutants *sy1* (SOSNIKHINA et al. 1992) and *sy9* (SOSNIKHINA et al. 1998) are asynaptic, resulting in high univalency at metaphase I. (Fig. 1a). Mutant *sy10* (FEDOTOVA et al. 1994) on the other hand, has indiscriminate synapsis that severely compromises recombination between homologues, and also results in high univalent frequencies at first metaphase.

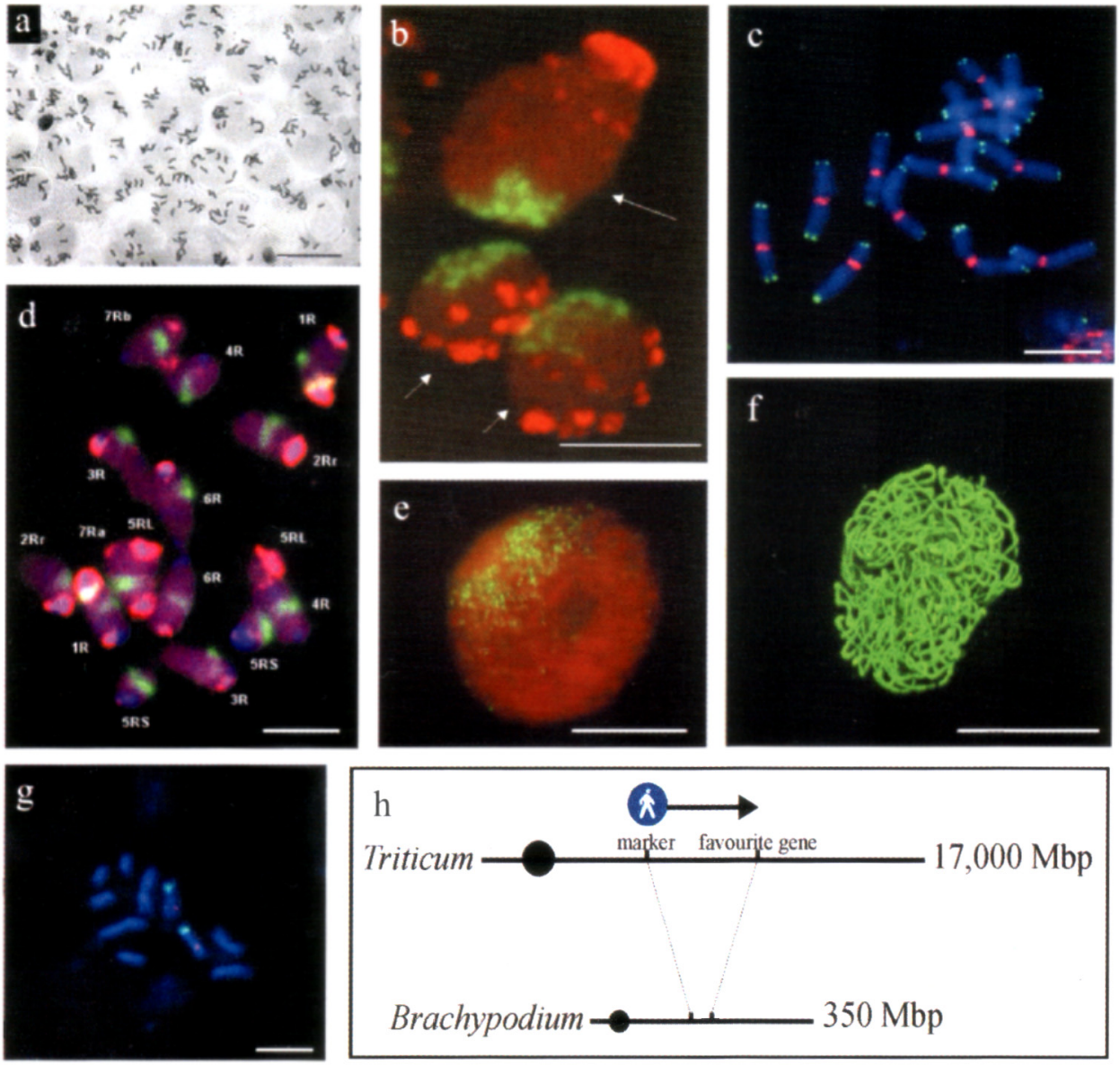

Figure 1: (**a**) High univalency at metaphase I in *syl*. Bar = 50 μm. (**b**) Bouquet formation of telomeres (red) and clustering of centromeres (green) at the onset of meiosis in a pollen mother cell of wild type (large arrow), and Rabl orientation of the two domains in tapetal nuclei (small arrows). Bar = 10 μm. (**c**) Somatic chromosomes labelled with centromeric sequence CCS1 (red) and telomeric array (green). Bar = 10 μm. (**d**) Optical stack of first metaphase chromosomes of *sy10* labelled with subtelomeric repeats pSC200 (red) and pSc250 (blue), CCS1 (green) and 25S rDNA (yellow). Labels indicate chromosome identities. Bar = 5 μm. (**e**) Early meiotic prophase nucleus of rye immunolabelled with anti- Rad51/Dmc1 antibody (green). Bar = 10 μm. (**f**) Optical stack of zygotene of wild type immunolabelled with anti-Asy1 antibody (green). Bar = 10 μm. (**g**) Two BACs (red and green) landed onto single loci of each arm of chromosome 2 of *B. distachyon*. Bar = 5 μm. (**h**) Diagram illustrating how the compact genome of *B. distachyon* may assist chromosome walking to genes of interest.

Early events in meiosis

Accurate recognition, pairing and synapsis of homologues are integral components of early meiosis, which ensure that homologues recombine and segregate in an orderly fashion. Since the mechanism and control of these processes are poorly understood at present, we embarked upon a comparative investigation of chromosome behaviour and nuclear architecture at early meiosis in wild type and mutant rye (MIKHAILOVA et al. 2001). Centromeres and subtelomeric chromosome domains were tagged with fluorescent probes and tracked through meiosis using fluorescence *in situ* hybridisation. The results showed that at premeiotic interphase the nuclear disposition of these domains was indistinguishable between wild type and the two mutants *syl* and *sy9*. However, at the onset of meiosis at leptotene, *syl* failed to form a characteristic cluster of telomeres (bouquet arrangement) and its centromeres dispersed prematurely from the Rabl orientation (Fig. 1b). Later on at zygotene telomeric aggregates failed to disperse in *syl*, and high frequencies of unpaired centromeres were recorded in *syl* and *sy9* as meiotic prophase drew to a close. The important observation here is that the mutant phenotype of *syl* manifests itself clearly before the stage at which synaptic failure is recognised. The inference is that a disturbance in the nuclear disposition of these chromosome domains could cause, or contribute to, asynapsis. As a refinement of this study, it is our intention to quantify associations of true telomeres (Fig. 1c) during this period of meiosis in wild type and mutant material undisturbed by squashing.

The indiscriminate synapsis of mutant *syl0* affords the possibility of finding an investigative route into the mechanism of homologue recognition. As a prelude to this study, multi-target FISH with confocal laser scanning microscopy has enabled the identification for the first time the complete chromosome complement of rye at meiosis (Fig. 1d). Not only has this allowed us to construct a meiotic karyotype of rye (JENKINS et al. 2005a), but it has also enabled the identification of chromosomes in all chiasmate and non-chiasmate associations at metaphase I. Surprisingly, 37% of the 35 bivalents observed in 78 optically reconstructed nuclei involved non-homologous chromosome associations. Furthermore, chromosome 4 was associated significantly more frequently than other chromosomes of the complement, suggesting an element of chromosome specificity in the way in which some individual chromosomes interact with others when pairing is compromised.

Sequencing meiotic genes

Comparative genomic investigations have shown that key structural and recombinogenic genes of meiosis have orthologues in very different organisms.

For example, a number of meiotic genes in the model plant *A. thaliana* have orthologues of known function in yeast (for review, see JENKINS 2003). Comparative approaches, especially with genome-validated models such as *A. thaliana* and rice, have the potential to isolate meiotic orthologues in rye. This approach has particular significance given the present dearth of sequence data in rye, and the current limitations of the genetic map of rye that precludes map-based cloning of meiotic genes. Conservation of structure of meiotic genes has enabled us to isolate and sequence two meiotic genes with key roles in molecular recombination (JENKINS et al. 2005a). RT-PCR on extracts from anthers and leaves of wild type and mutants, using degenerate primers based upon all known plant protein sequences, has allowed us to clone the rye orthologues of the structurally related *RAD51* and *DMC1* genes. Sequencing the central 600 bp reading frame of these clones has revealed a 98% identity to barley Dmc1 protein, and a 95% identity to maize Rad51B protein. Interestingly, comparison of the products of wild type and *syl* has shown post-transcriptional processing errors in the latter, such as small structural rearrangements and retention of introns. It is likely that these disrupt protein function, and may be a contributory factor in the absence of Rad51/Dmc1 protein in the *syl* mutant (see below).

Analysis of meiotic proteins

The conservation of structure of recombinogenic and structural proteins allows the immunolocalisation of meiotic proteins in rye, using antibodies to orthologous proteins of other organisms. We have begun a systematic assay using this approach of the temporal and spatial expression of proteins with key roles in meiosis, the eventual aim being to complete phenotypic "identikits" for each rye mutant. For example, the polyclonal antibody to Rad51/Dmc1 of tomato recognises its epitope in wild type rye and *sy9* (Fig. 1e), but not in *syl* (JENKINS et al. 2005a). The antibody to the synaptonemal complex (SC) – associated structural protein Asy1 from *A. thaliana* (CARYL et al. 2000), which incidentally corresponds to Hop1 of yeast (HOLLINGSWORTH, BYERS 1989), highlights chromosome cores from leptotene through to diakinesis of meiosis in wild type rye (Fig. 1f), but is absent or disorganised in 90% of pollen mother cells of *sy9*. We are undertaking simultaneous immunolabelling with two antibodies to different proteins, to determine particularly how structural and recombinogenic proteins interact during meiosis. Also, it is our intention to improve the resolution of localisation, particularly with respect to the SC, using immunogold labelling and electron microscopy. The SC itself can be considered a paradigm for the assembly and interaction of proteins during meiosis, so we are making a special effort to isolate and characterise it in rye. The SC is relatively easily viewed, iso-

lated and purified in rye, and could provide useful substrates for proteomic analysis using 2D gel electrophoresis and mass spectrometry (JENKINS et al. 2005a). There is, therefore, the potential for exploiting rye to isolate novel proteins associated with the SC, particularly those that may be ephemeral or of low abundance.

Raising a "bridge"

The value of *A. thaliana* and rice as models for functional genomics in plants is undisputed and cannot be overstated. However, given the relative ease nowadays for the building of functional genomic infrastructure for other organisms, it may be prudent to evaluate whether or not other organisms have niche or specialist roles to play alongside the mainstream models. This consideration is particularly apposite if the best model does not adequately represent the biological system under scrutiny. For example, the model may be too distant phylogenetically, or may not have the same repertoire of genes, which may preclude map- or sequence-based cloning due to breakdown in microsynteny. As a consequence, the model may actually have only limited utility in terms of functional genomics and gene discovery.

DRAPER et al. (2001) and FOOTE et al. (2004) recognised that the gulf between our current plant models and the temperate cereals and grasses may be so great as to confound or complicate comparative approaches. Alternatives were therefore sought, and our attention focussed upon the genus *Brachypodium*. One species of this genus, *B. distachyon*, has particularly desirable features that may confirm its place as a useful bridge species between the two model plants and the group of temperate grasses it represents. Since its official launch (DRAPER et al. 2001), considerable effort has been made to evaluate its usefulness and explore ways in which it could be used in scientific investigations (JENKINS et al. 2005b). We reported to the "grass fraternity" at the last PAGEN meeting how we were building up the molecular cytogenetic infrastructure of this new model grass (JENKINS et al. 2003). Since that meeting, we have made strides in our understanding of the karyotype of this species, and have determined that its polyploid series is in fact based upon hybridisation of related species (HASTEROK et al. 2004). Moreover, two bacterial artificial chromosome (BAC) libraries have been constructed in the model diploid genotypes ABR1 and ABR5 ($2n = 2x = 10$), which are currently being assayed for microsynteny to grass relatives through DNA sequencing and the landing of BACs onto chromosome arms (Fig. 1g).

B. distachyon has an extremely compact and economical genome (350 Mbp), with little repetitive DNA to distantly space genes along chromosomes. Given

sufficient colinearity between this species and its relatives, it is plain to see that a marathon chromosome walk from a marker to favourite gene in a cereal could translate into a short stroll in *B. distachyon* (Fig. 1h), and significantly improve the chances of isolating a gene of interest.

Clearly, we have not yet realised the potential or importance of *B. distachyon* in this context. We are perhaps at a threshold, where we need to decide if this species is important enough to be backed by international collaboration. If this species were to be adopted by the international community, there is the likelihood that a draft genomic sequence could be available very quickly. Indeed, given the same resources as the Human Genome Project, which of course is an unlikely scenario, this could be published in one to two months from start to finish!

References

Caryl A.P., Armstrong S.J., Jones G.H., Franklin F.C.H. (2000). A homologue of the yeast *HOP1* gene is inactivated in *Arabidopsis* meiotic mutant *asy1*. Chromosoma 109: 62-71.

Draper J., Mur L.A.J., Jenkins G., Ghosh-Biswas G.C., Bablak P., Hasterok R., Routledge A.P.M. (2001). *Brachypodium distachyon*. A new model system for functional genomics in grasses. Plant Physiol. 127: 1539-1555.

Fedotova Y.S., Bogdanov Y.F., Gadzhiyeva S.A., Sosnikhina S.P., Smirnov V.G., Mikhailova E.I. (1994). Meiotic mutants of rye *Secale cereale* L. II. The nonhomologous synapsis in desynaptic mutants *sy7* and *sy10*. Theor. Appl. Genet. 88: 1029-1036.

Foote T., Griffiths S., Allouis S., Moore G. (2004). Construction and analysis of a BAC library in the grass *Brachypodium sylvaticum*: its use as a tool to bridge the gap between rice and wheat in elucidating gene content. Functional and Integrative Genomics 4: 26-33.

Goff S.A., Ricke D., Lan T.H., Presting G., Wang R., Et A. (2002). A draft sequence of the rice genome (*Oryza sativa* L. ssp. *japonica*). Science 296: 92-100.

Hasterok R., Draper J., Jenkins G. (2004). Laying the cytotaxonomic foundation of a new model grass, *Brachypodium distachyon* (L.) Beauv. Chrom. Res. 12: 397-403.

Hollingsworth N.M., Byers B. (1989). *HOP1*: a yeast meiotic pairing gene. Genetics 121: 445-462.

Jenkins G. (2003). Unfolding large-scale maps. Genome 46: 947-952.

Jenkins G., Hasterok R., Draper J. (2003). Building the molecular cytogenetic infrastructure of a new model grass. In: Z. Zwierzykowski, M. Surma, and P. Kachlicki (eds.), Application of Novel Cytogenetic and Molecular Techniques in Genetics and Breeding of the Grasses, 77-84. Institute of Plant Genetics, Polish Academy of Sciences, Poznań, Poland.

JENKINS G., MIKHAILOVA E.I., LANGDON T., TIKHOLIZ O.A., SOSNIKHINA S.P., JONES R.N. (2005a). Strategies for the study of meiosis in rye. Cytogenet. Genome Res. 109: 221-227.

JENKINS G., MUR L.A.J., BABLAK P., HASTEROK R., DRAPER J. (2005b). Prospects for functional genomics in a new model grass. In: D. LEISTER (ed.), Plant Functional Genomics, 305-325. Haworth Press.

KLECKNER N. (1996). Meiosis: How could it work? Proc. Natl. Acad. Sci. USA 93: 8167-8174.

MIKHAILOVA E.I., SOSNIKHINA S.P., KIRILLOVA G.A., TIKHOLIZ O.A., SMIRNOV V.G., JONES R.N., JENKINS G. (2001). Nuclear dispositions of subtelomeric and percentromeric chromosomal domains during meiosis in asynaptic mutants of rye (*Secale cereale* L.). J. Cell Sci. 114: 1875-1882.

ROEDER G.S. (1997). Meiotic chromosomes: it takes two to tango. Genes and Development 11: 2600-2621.

SOSNIKHINA S.P., FEDOTOVA Y.S., SMIRNOV V.G., MIKHAILOVA E.I., KOLOMIETS O.L., BOGDANOV Y.F. (1992). Meiotic mutants of rye *Secale cereale* L. I. Synaptic mutant *sy-1*. Theor. Appl. Genet. 84: 979-985.

SOSNIKHINA S.P., KIRILLOVA G.A., MIKHAILOVA E.I., SMIRNOV V.G., FEDOTOVA Y.S., BOGDANOV Y.F. (1998). Genetic control of synapsis in rye *Secale cereale* L.: The *sy9* asynaptic gene. Russian J. Genet. 34: 1278-1285.

SOSNIKHINA S.P., MIKHAILOVA E.I., TIKHOLIZ O.A., PRIYATKINA S.N., SMIRNOV V.G., DADASHEV S.Y., KOLOMIETS O.L., BOGDANOV Y.F. (2005). Meiotic mutations in rye *Secale cereale* L. Cytogenet. Genome Res. 109: 215-220.

THE ARABIDOPSIS GENOME INITIATIVE (2000). Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature 408: 796-815.

YU J., HU S., WANG J., WONG G.K., LI S., ET A. (2002). A draft sequence of the rice genome (*Oryza sativa* L. ssp. *indica*). Science 296: 79-92.

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