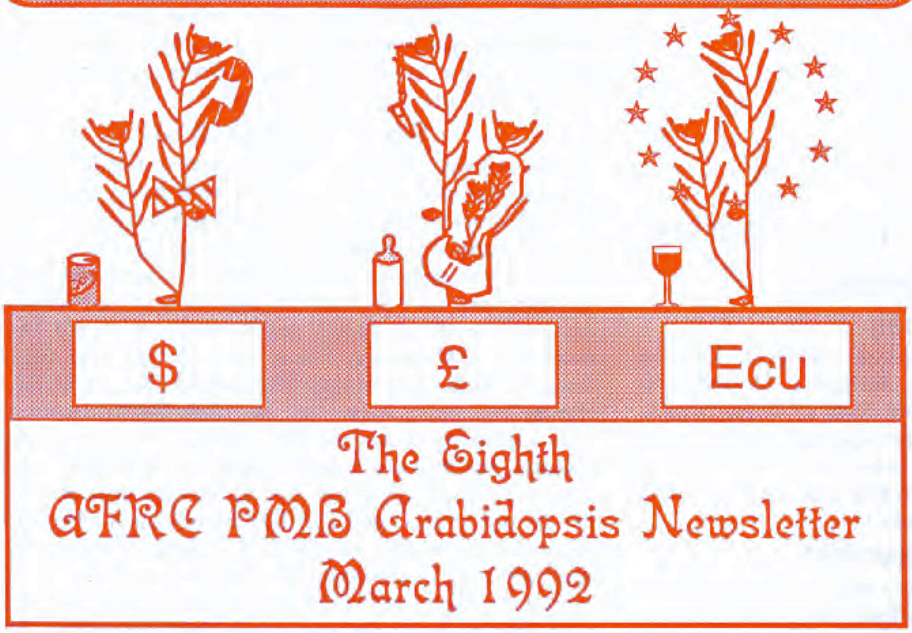
Thale of the Century



The Eighth AFRC PMB Arabidopsis Newsletter March 1992

In This Issue:

Plus – attached:

How to Reach Us

Please send all contributions to this newsletter by either, e-mail to: ARABIDOPSIS@UK.AC.AFRC.JII (or ARABIDOPSIS@ JII.AFRC.AC.UK for some non-U.K. e-mailers), or on disk. Mac disks are ideal, but we can import MS-DOS (IBM) too. With IBM output, please send the file on either a 3½" (preferably) or 5¼" disk with the file in wordprocessor format and as a text-only (ASCII) file. Whatever the disk, label it with the type and version of wordprocessing programme used. Also enclose a printed copy and ensure that the disk and originating machine are virus-free. Disks will, of course, be returned. Further details about communicating *via* computer are given in the second newsletter (Arabidian Notes). File transfer by modem is also available for the *cognoscenti*.

Owing to the AFRC PMB *Arabidopsis* Programme's July meeting, there will be no summer newsletter. The deadline for the next newsletter is, therefore:

Monday, 5 October, 1992.

Thale of the Century: the eighth AFRC PMB *Arabidopsis* Newsletter, March 1992.
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From Mary Anderson...

THE NOTTINGHAM *ARABIDOPSIS* STOCK CENTRE

New Faces

THE STOCK CENTRE TEAM has two new members. Dr Igor Vizir has joined us from Kishinev, Moldavia. He has been very busy characterising the T-DNA lines of Csaba Koncz. He is also testing a strategy for generating deletion mutants. See his report below. Our other new face is Tania Perehinec, a research assistant who works part-time for me and does a valiant job with the plants.

The Kranz Collection

SINCE THE LAST newsletter article, we have been very busy characterising the Form Mutants from the Kranz Collection. This has turned out to be a very interesting exercise and will be of particular interest to those of you working on development. As a taster, the collection contains: a whole range of dwarf mutants, some of which are only 1 cm tall; chlorophyll mutants that are also dwarfed; plants where the leaves of the rosette are orientated like a Catherine wheel in a spiral fashion. There are also plants which exhibit varying degrees of marginal leaf rolling. In addition, there is a range of mutants where the first leaves are spotted and then fully green in later development, or where the margin of the leaf is paler in colour as if the edge is outlined.

We nearly have single-seed descendants for each of the form mutants, except where the plants are segregating steriles, in which case we have attempted to catch heterozygotes. We have photographed most of the mutants. The pictures will be a source of reference when trying to describe the nuances that distinguish some of these lines. In the same way that Inuit have a hundred words for snow I need a similar number of descriptors for all the shades of green that I have seen! The hope is that this pictorial information can be scanned into the Mac and incorporated into the new database systems, which are being developed in the States -- (see the database special), so that they can be looked at as you scan through the electronic seed catalogue. We do not intend to bulk the form mutants directly. We will wait to see what the interest will be for individual lines and bulk these as necessary. I am afraid you will have to wait until the next newsletter for the descriptions of the form mutants to be collated. However, I am happy to answer any general questions or requests about the collection, before the release of the detailed stock descriptions.

Besides taking photographs of the form mutants, we have started to get together herbarium specimens of the plants. This collection will be held at Nottingham and will be available for anyone who wishes to come and view it, although from the experience of Prof. Kranz, photocopies of these mutants give a very fair representation of the mature plant.

As for the rest of the Kranz collection: colour mutants, biochemical mutants, marker lines and ecotypes will in turn be characterised over the coming months. We intend to produce a new seed list by the end-of-programme conference in July, which give full details.

Seed Storage Alert

WE ARE NOW well into the third year of the AFRC PMB Programme and so a lot of you will have seed that is probably over two years old and has just been stored in the lab. **Beware**, it is possible that this seed has lost a lot of its viability, especially if it has been stored in a relatively humid environment, such as in the fall-out zone of pressure cookers and the like. Bernie and I went on a fact finding mission to Wakehurst Place, part of the Kew Gardens research outfit, near Hemel Hempstead. Roger Smith and Simon Linington spent the day showing us round their very sophisticated seed-storage facilities, which we hope to emulate, but on a much smaller scale, at Nottingham. Their take-home message about storing *Arabidopsis* is that if it can be put at 15°C and 15% RH immediately after harvest and then taken down to 5% RH and stored at -20°C, the seed will keep indefinitely. Most of you will have to compromise on these conditions, but the main criterion for long term storage is for the seeds to be dried correctly. In the past we have recommended silica gel to dry the seed and in general this should be O.K. However, we are unsure about the actual water content that this establishes in the seed. We are continuing trials to establish how to store seeds in the long term. If you have seed that you wish to put into long term storage, grow up a fresh batch and store that.

Nottingham Links With Ohio

DURING NOVEMBER and December we sent the Ohio *Arabidopsis* Resource Centre all our stock lines, which they are bulking. They will shortly be geared up for distribution. They are also going to help with the characterisation of the Kranz collection before they too start to characterise the American collection of George Redei.

The Ohio team are also very busy establishing the *Arabidopsis* Information Managemant System (AIMS), (see the database special), which we will be soon feeding with all our stock details. This will be a Stock Centre based database that anyone will be able to access through the electronic mail system, or by ftp. This will make a tremendous difference to the administration of the Nottingham Stock Centre, as AIMS should become a master database for both Centres. AIMS will carry the most up-to-date seed catalogue (entries to the electronic seed lists could occur weekly) and will have an automated ordering facility. Nevertheless, we shall continue to produce hard-copy lists as we are well aware that many Europeans do not have easy access to the electronic mail system. However, cost and time constraints dictate that we can only produce one hard copy seed list per year.

Recombinant Inbreds Come To Nott'm

PABLO SCOLNIK of Du Pont, Wilmington has kindly donated approximately 100 recombinant inbreds (RIs) to both *Arabidopsis* Stock Centres. These lines have been produced by crossing WS and W100F. A good background reference for recombinant inbreds is the review article Burr & Burr (1991, TIG 7, 55-60). The key reference for these particular lines is Reiter *et al.* (1992)

Proc Natl Acad Sci (USA) 89 (in press). Practical instruction for using this collection to map genes will be published in the *Arabidopsis* handbook that Csaba Koncz is producing. We do not have the software programme for processing the mapping information, but Pablo will be happy to deal with this information, if you send him your data. His address is: Pablo Scolnik, E.I. Du Pont de Nemours and Company, PO Box 80402, Wilmington, Delaware 19880 0402, USA. Fax: 302 695 8480.

If you want to receive these lines or require further information please contact me directly.

Please note that Clare Lister & Caroline Dean have also produced 300 RIs from a Landsberg/Columbia cross. RFLP and RAPD markers will be mapped onto a mapping population of 100 of these lines. This seed is now being bulked and should be ready for distribution by **October**.

T-DNA Lines

Ken Feldmann has very kindly organised to donate his T-DNA lines to both Stock Centres sometime in February, so by the time the Newsletter hits the bookshelves we should also have these materials. Ken will send seed sufficient for 100 screens. Before you reach for the phone to place an order, perhaps you could get together with colleagues at the same site as you, or close by, who could share the screening process. This will probably be the most cost effective way of conducting screens and will make the best use of these valuable lines, a not unlimited resource.

If you want to receive these lines or require further information please contact me directly.

Igor Writes...

We are characterizing about 400 T-DNA tagged lines from Csaba Koncz. We are at the stage of sibling harvesting from each plant of a family. The next goal is to find plants that are homozygous for T-DNA insertions. This will simplify future work with this material. Also, we are planing to map the most interesting mutants, as this will simplify subsequent genetic analysis.

In addition, we are starting to characterise and bulk the collection of mutants and races which I have brought from Moldavia – more details in the next report.

We have also been developing a method based on the gamma-irradiation technique for the induction of deficiencies and deletions, as a tool to clone genes rapidly. We have chosen as our models the *gl1* (chr. 3) and *ttg* (chr. 5) genes. At the moment, we have isolated several new mutations of these genes; potential *ms1* and *fg* mutants have also been observed. The next and very important step, will be to determine whether new mutations are really due to deletions or deficiencies, and if so, of what size these are.

More Information From

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From Jeff DangL.

BRIDGE DNA Stock Center, Köln

"Them as gives, gets." Was that Dickens, Trollope, or Ronald Reagan? I can't recall. But it is apt as an intro to this update. I made, I thought, as down-home a plea as possible several newsletters ago, saying that the utility, and long-term staying power, of a DNA resource centre would be entirely investigator driven. Thus far, my plea has gone rather unheeded. Again, please remember that the future of a European long term DNA stock centre for Arabidopsis is still in your hands. Please deposit your phage libraries, and feel free to take advantage of the kindness of your colleagues by requesting things from us. OK, enough grovelling. What we have on hand is:

RFLP probes: Meyerowitz backbone set of 90, plus around 40 more from Elliot's lab. These are supplied as phage lysates. We have sent them, so far, to six groups, none from the BRIDGE or AFRC programmes.

YAC libraries: EW and EG banks. We have sent these out twice, once to a BRIDGE participant.

Genomic phage libraries: Col-0 in λGEM II; La-er in λDASH II Neither of these was deposited by a BRIDGE participant.

cDNA phage libraries: from La-er flowers in λZAP II; from total sterile Col-0 in λgt10; and from "aerial parts" Col-0 in λZAP. None of these was deposited by Bridge participant.

Besides sending out the RFLP probe set and the YAC libraries, we have filled quite a number of phage library orders. Oddly, only one came from a BRIDGE or AFRC participant. Thus, were are surely serving some needs, of other Europeans, some folks in the US, and even Korea. Yet motivating the groups we were ostensibly to serve has proven nigh on impossible.

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Please Note:

Owing to the PMB Arabidopsis Conference being held in July, there will be **NO** newsletter this summer.

The next newsletter will be in the autumn. The deadline is:

MONDAY, 5TH OCTOBER.

AFRC PMB *Arabidopsis* Conference

The end-of-term finale for the grants in the original AFRC PMB *Arabidopsis* Programme will be held at the University of East Anglia, Norwich, from Monday, 13 July to Wednesday, 15 July inclusive. If you are attending (at least one person is obliged to come from each grant), please fill in the registration form accompanying this newsletter and FAX (to 0603-505725 or 0603-56844) or mail it to the ACM as soon as possible.

As with the previous meeting, held in Nottingham during December 1990, the conference is nominally limited to PI's plus one other for each grant. It is expected, however, that there will be a few more places available. Will PIs please indicate on the registration form the names of extras they would like to attend? If you aren't a PI or "plus one" and came last time (or have replaced someone who did) and wish to come, please fill in a form at this stage. It is expected that there will be spaces available for all those who attended the Nottingham conference.

Please fill in the registration form and return it to the A.C.M. a.s.a.p.

The provisional timetable is as follows. Registration will take place on Monday (13th) morning and during lunchtime. The first session will take place in the afternoon. In the evening, there will be a short session at John Innes, followed by a poolside barbeque. On Tuesday (14th), there will be sessions all day, followed by the Conference Dinner in the Sainsbury Centre. On Wednesday (15th) there will be the final session in the morning and, after lunch, visits to the John Innes Centre for those interested.

As with the Nottingham meeting, non-*Arabidopsis* grant holders from the U.K. may attend, but must pay their way. This will be approximately £100 inclusive of all meals and accommodation from Monday lunchtime to Wednesday lunchtime. Please note, to avoid saddling the ACM with even more of an administrative nightmare, that for those paying this will be a fixed price. There will be no reduction for, *e.g.*, not attending the conference dinner.

For grant holders, all meals and accommodation will be provided. Reasonable travel costs, preferably by train or sharing a car, to and from Norwich will be refunded. Airfares will not be paid. For participants coming long distances, accommodation may be provided on the Sunday and Wednesday nights. Please note, however, that the conference has been structured to allow most participants time to travel on Monday morning and Wednesday afternoon or evening.

As with the previous meetings, the format will be that a representative from each grant gives a short talk. Those non-grant holders who wish to attend must bring a poster. For grant-holders wishing to supply more information, poster space will also be available.

In line with the meetings of the "General" half of the PMB Programme, a book of abstracts will be produced to be distributed at the meeting. These abstracts will be instead of the summer newsletter. They will not be in the form of a newsletter nor will they be distributed to the general newsletter readership. Abstract forms will be sent out, together with further details of the meeting, sometime this Spring.

The deadline for the next full newsletter, which will be the last of PMB I, is Monday, 5 October.

If you did not receive a booking form and would like to attend, please contact the ACM.

From Fred Lehle...

Fast Neutron Irradiated *Arabidopsis* Seed

Lehle seeds expects to begin harvesting fast-neutron irradiated M2 seed in the last week in February. M2 seed will be available in three backgrounds, Columbia, Landsberg *erecta*, and RLD and will be bulked in parental groups of about 1000 M1 parents.

The irradiation dosage of the seed to be offered was 60 Gy and was performed by the IAEA in Vienna Austria. Seeds were irradiated dry in paper envelopes. I have grown out a small sample of these seeds (Landsberg *erecta* background only) and have made the following preliminary observations.

Establishment is about 15% of untreated controls. I define establishment as the percent of seeds which produce a fertile M1 parent. I estimated this by germinating the seeds on nutrient agar plates and then transferring all viable seedlings to potting mix.

I also selected four siliques each from fertile M1 parents and counted the number which showed segregation for albino embryos. It is my understanding that albino embryos are rarely seen in unmutagenised material. The work is still in progress, but 16 out of 78 (20%) of the fertile M1 parents exhibit segregation of albino embryos among green embryos.

I will place a notice on the bulletin board as soon as M2 seeds are available for shipment. Pricing will be provided at that time also. If you wish to be contacted personally as soon as these fast neutron irradiated seeds are offered, kindly send a card, letter, or FAX with your name and address to the following location: Lehle Seeds 6531 North Camino Katrina Tucson AZ 85718 USA. Phone: +602-544-0733 FAX: +602-797-9009 (This is our new fax number). LEHLEF@EDU.ARIZONA.CCIT

Philip Horsnell

It is with deep regret that we report the death of Philip Horsnell, the post-doc on Christine Raines's grant (Genetic analysis of regulatory factors determining the deveolpment of the photosynthetic apparatus of plants). Many of you will know him through his production and generous distribution of a successful *Arabidopsis* cDNA library. A lasting memory of him for many of us is of his *tour de force* presentation at the Nottingham *Arabidopsis* Meeting in December 1990. Called up at the last-minute, he spoke without the aid of notes, slides or overheads and kept the whole audience spellbound about the trials and tribulations of using kits to make a cDNA library.

Our sympathies are extended to his wife, family, friends, and colleagues.

From Sue Albini...

Synaptonemal complex spreading: an ultrastructural approach to chromosome analysis in *Arabidopsis thaliana*.

The main aim of this project is to perform a detailed analysis of prophase I of meiosis in Arabidopsis by studying the synaptonemal complex (SC). A method has been developed by which all stages of prophase I, leptotene, zygotene, pachytene and diplotene, can be analysed. Despite the arduous nature of these experiments, over 150 prophase I nuclei have been photographed in the electron microscope. The nuclei range from those in which there is little or no homologous pairing to those in which the maternal and paternal homologues are fully paired. Prophase I in Arabidopsis proceeds in much the same way as in other higher plants. Pairing initiates at or the ends of the bivalents, but subsequently there are multiple sites of SC initiation. Pairing progresses from the telomeres towards the centromeres and the peri-centromeric regions are the last to pair.

At pachytene, there are five synaptonemal complexes. SC associated structures such as centromeres and nucleoli are often well preserved. This aids identification of the SCs and karyotyping. The appearance of the nucleoli is variable. In some nuclei it is difficult to identify the nucleolus yet in others an enormous nucleolus is observed. The variation is to some extent dependent on genotype, but other factors including technical and stage effects probably contribute too. Nucleoli often fuse so that although sometimes two nucleoli are seen most often only one is observed. However, it is still possible to identify the SCs that carry the nucleolus organising regions by their association with the nucleolus. In many nuclei, the nucleoli are astonishingly large, much larger relatively and absolutely, than those in surface spread SCs of other higher plants, *e.g.*, rye, onions and barley.

Other stages of meiosis, *e.g.*, metaphase I, have also been examined. A preliminary study of flo 10 (a floral mutant from George Haughn), has shown that the mean chiasma frequency is about ten. Given that one chiasma equals fifty centimorgans, the genetic map length of Arabidopsis calculated on this limited data is five hundred centimorgans. Most bivalents have two chiasmata and some have one or three. It is possible, with some confidence, to determine whether or not a chiasma is nearer the end of a bivalent (distal) or nearer the centromere (non-distal). In the Arabidopsis material examined over sixty percent of the chiasmata were distal. There is no extreme localisation of chiasmata, but distal regions appear to be preferred.

> "In Arabidopsis...over 60% of the chiasmata were distal."

Prophase I in chromosome mutants, *e.g.*, polyploids and inversion heterozygotes, is also being investigated. The polyploids have larger nuclei, but this is offset by their only having very few pollen mother cells (PMCs). The study of meiosis in Arabidopsis polyploids is only for the truly dedicated or the slightly mad.

That Arabidopsis PMCs have a mean SC length of around 150μm has provoked a lot of interesting speculation on the rôle of the SC in meiosis. *(See Sue's report on the meiosis meeting in Wageningen on page 25 -- ACM.)*

In addition to the main thrust of this project, I am attempting to apply the techniques of molecular cytogenetics to the study of meiosis in plants. In particular, DNA-DNA *in situ* hybridisation to SC preparations. As reported previously, *in situ* hybridisation of ribosomal DNA to rye SCs detected with fluorescein, has proved successful. I have repeated this several times. Using some of my rye spreads, Trude Schwarzacher in Norwich has successfully used a different probe (pSc119.2) which carries a 120bp repeat found in rye heterochromatin. Using the probe pTa71, which contains rDNA derived from wheat, I have applied this technique to meiocytes of Arabidopsis.

In the somatic cells derived from the floral tissue four sites of hybridisation light up, which is consistent with the presence of two pairs of nucleolus organising chromosomes. In the PMCs one distinct site is visible, which is probably because the two nucleoli often fuse so that the two nucleolus organising regions are likely to be in close proximity. In one experiment, the cells were entering metaphase I, the nucleolus had disappeared by this stage and five condensed bivalents were visible. In these nuclei, two sites of hybridisation on two different bivalents were seen.

The plan for the next few months is to carry on with the *in situ* experiments using both Arabidopsis and rye SC preparations and a range of different probes, *e.g.*, Arabidopsis telomere sequences obtained from Eric Richards. In addition to this the task of obtaining SC spreads of Arabidopsis, for *in situ* experiments, electron microscope observations and hopefully *in situ* at the EM level, goes on.

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From Ken Buck...

A novel approach to the isolation of origins of plant DNA replication using *Arabidopsis* as a model system.

We now have obtained transgenic plants containing the NEO and HYG ORI vectors. The plants containing the NEO vector are being tested for the presence of replicating circles. The HYG ORI vector has been shown to recombine to form an intact hygromycin resistance gene and DNA extracts from hygromycin-resistant calli have been shown to contain extrachromosomal DNA which hybridises to hygromycin gene probes.

Having demonstated that our vector system works with a virus origin of replication, we can now investigate Arabidopsis origins. There is good evidence that in yeast and some other organisms, an origin of DNA replication is located in the non-transcribed spacer regions within the ribosomal DNA repeat units. Therefore, we have obtained Arabidopsis ribosomal DNA clones from Elliot Meyerowitz & Inge Unfried. Fragments of ➳

these clones are being cloned into our ORI vectors. We also plan to use a series of overlapping Arabidopsis cosmid clones, on the basis that Arabidopsis origins have been shown to occur, on average, every 25-30 kb.

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From Neil Butt..

Cell cycle control genes in *Arabidopsis.*

Sequencing of our UBCa gene now appears to be complete. Comparisons with other UBC proteins shows considerable similarities with all classes so far identified, especially with UBC4 of *S. cerevisiae.* This gene is a central component of a major proteolytic pathway of *S. cerevisiae.* Several lines of evidence suggest that not only controlled gene expression, but also post-translational control of protein levels by selective breakdown, is important for cell differentiation and developmental processes. This has been shown for the bicoid protein, a transcription factor present in the embryo, in *Drosophila* (Driever & Nusslein-Volhard, 1988) and it is known that ubiquitin-dependent proteolysis seems essential for developmentally programmed cell death (Schartz *et al.*, 1990).

We suggested in our last item that the UBCa gene appears to be expressed at low levels. This has been confirmed by the absence of any signal on northern blots that contained total RNA from young plants, old plants, leaves, roots, flowers, seeds and callus. We have proceeded to obtain mRNA from some of these tissue samples and are probing these to observe any transcripts.

This material has also been used to produce cDNA for construction of cDNA libraries. The low level of expression of this gene has made it difficult to find the relevant cDNA from our existing libraries. Initial screens were performed with the 3' region of the gene as a probe, which appears to confer type specificity to this group of proteins. This, however, has not been successful so far. We are now using the 5' region of the gene which has been shown to be much more conserved (several bands were obtained when hybridised to Southern blots of genomic DNA cut with a variety of restriction endonucleases). This will allow us to identify our UBC cDNA and any other related sequence.

In order to observe expression levels and expression patterns of this protein, UBCa promoter/GUS fusions have been constructed and used to infect root material. The *Agrobacterium* infection appears to have been very successful with plenty of tissue showing regeneration on selective medium. Some of this tissue has regenerated into small plantlets and GUS assays will be performed on this material in the near future. In view of determining a functional role for this protein, we are interested in obtaining an *Agrobacterium* vector with an inducible promoter, or a flower specific promoter. This is to enable expression of the protein at controlled stages of the life cycle so if any deleterious effects do occur we can allow the plants to regenerate from callus first. If anyone has such a vector or promoter that we can manipulate we would be very interested to hear from you. *(See Alison Smith's report -- ACM ?)*

The isolation of cdc22 and suc22 is still in progress. Initial sequencing of suc22 cDNA clones was not hopeful, but subsequent analysis has generated several new clones which appear to be much more encouraging. These are being sequenced, and screening of a genomic library is underway. Screening for cdc22 has not been so successful, but genomic libraries are still being probed. These genes possess promoters induced by the cell cycle or DNA damage in yeast, so if plants also contain these genes we aim to construct GUS fusions to observe the control of expression.

Several other aspects of the cell cycle are still being investigated. Western blotting with suc1 antibodies has given a band at 18kDa, which appears to be twice the size of the human protein (9kDa) and larger than the *S. pombe* protein (13kDa). Further analysis is continuing on this protein. We have also recently started to look at the control of gene expression, setting up gel-retardation assays to identify control elements. This work is very recent and we do not have much to report.

Away from the cell cycle, we have constructed several *Agrobacterium* plasmids containing mitochondrial genes (the other area of interest in our laboratories). Constructs containing glycine decarboxylase, alternative oxidase and heat shock protein genes have been made. Of these only the glycine decarboxylase has been used to infect plants, but these are at too early a stage to assess the level of success. The other genes are being cloned into the BIN400 series of vectors, as opposed to JE188 which was previously used, because our group finds this series much easier to manipulate.

Driever, W., & C. Nusslein-Volhard (1988) *Cell* 48, 1035-1046.
Schwartz, L. M., A. Myer, L. Kosz, M. Engelstein, & C. Maier. (1990) *Neuron* 5, 411-419.

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From Jeremy Carmichael & Jim Murray...

Molecular identification and analysis of genes involved in plant development and growth control.

Random checks on our first Arabidopsis callus cDNA library reported in the October newsletter revealed a large number of empty clones (plasmids that had religated without inserts). However, we had been able to produce cDNAs with sizes ranging from 0.5-7kb as judged from the first strand synthesis. We concluded that some linkers and adaptors were contaminating the prepared cDNA and were ligating preferentially to the vector. The problem lay in the columns supplied with the kit for size selecting cDNA which had allowed smaller fragments to leak through. After informing the company we were given a replacement kit and have decided to use an alternative type of column for doing the size selection step (Chromaspin-400 columns from Clontech).

We have prepared a second library in the *E. coli* expression vector,

pSPORT (Gibco BRL). Of the clones analysed, 70-80% contain inserts and we hope to improve on this using multiple rounds of column purifications. The library has approximately 300,000 independent recombinants. We can hopefully increase the sizes of our yeast expression libraries by scaling up the cDNA synthesis.

One of our students has begun to prepare plasmid from John Mulligan's λYES library using its cre-lox excision system and is involved in experiments to optimise yeast transformation protocols with this and with a second budding yeast expression library.

Sequencing of the putative B-type cyclin clones isolated by screening the λYES library with probes generated by PCR continues. One large 2.4 kb clone is being used as a probe on northern blots to check if it is full length before being subcloned to test for complementation in yeast.

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From George Coupland...

Isolation of the flowering-time gene *fg*.

Mutations at the *fg* locus cause late-flowering under long day conditions, but do not affect flowering-time under short days. As described in *A-rap-a-bop-sis* (p. 15), we are chromosome walking to this gene. Located on chromosome 5 at 16.4cM, *fg* is very near to *tt*-4 (15.1cM). Since *tt*-4 is caused by a mutation in the chalcone synthase gene, we (Jo Putterill, Frances Robson & Karen Ingle) could use this as a starting point for our walk.

We have used the YAC libraries constructed by Erwin Grill & Chris Somerville and one made by Eric Ward to try to build a physical map of the region between *fg* and chalcone synthase. So far, we have two contiguous segments of cloned DNA: one of approximately 650kb includes marker CHS and 6833 while the second is around 1100kb and contains markers CIT1243 and 5962.

In the last three months we have mainly concentrated on trying to place *fg* on these contigs by RFLP mapping. To isolate recombinants closely linked to *fg* on the *tt*-4 side, we made a Landsberg *erecta*, chromosome marked with *fg* and *tt*-4. This line was crossed to Niederzenz and recombinants were identified in the F_2 in which cross-overs had occurred between the two mutations. We have identified, and confirmed 12 of these in the F_3 generation. In a similar experiment, we screened an F_2 population (provided by Maarten Koornneef) for recombinants between *fg* and *alb*-2 (located at co-ordinate 17.2cM on chromosome 5). We found and confirmed four recombinants between *fg* and *alb*-2.

"This has located *fg* within a 350kb region defined by three YAC clones."

Our most exciting results have come from mapping several probes from different locations within the CIT1243/-5962 contig relative to the break-points in eight *fg-tt*-4 recombinants and three *fg- alb*-2 recombinants. This analysis has located *fg* within this contig and further work showed that it is within a c. 350kb region defined by three YAC clones. It should be possible to narrow this down further with the recombinants available to us since one break-point on the *alb*-2 side and 2 on the *tt*-4 side fall within the 350kb region. We are now trying to obtain more probes within this region by pursuing the isolation of some YAC end-probes, which have proved difficult to make, by isolating λclones that hybridise to other end probes with which we couldn't identify polymorphisms and by starting to sub-clone the 3 YACs into cosmid vectors.

Transposon tagging.

June Swinburne & Lluis Balcells have made and studied 19 *Arabidopsis* transformants containing fusions of the octopine synthase, nopaline synthase, CaMV35S and *Ac* promoters to the *Ac* transposase ORF. These transformants have been examined both for their ability to drive excision of *Ds* from a streptomycin resistance gene and for the abundance of the transposase mRNA. The latter was measured using an RNase protection method. In general, high levels of mRNA correlated with frequent excision of *Ds*. For example, the most active CaMV35S transposase transformant contained approximately 23 times more mRNA than the least active transformant with the same construct. The one with least *Ac* mRNA showed little transposition activity while the one with most mRNA promoted high frequencies of somatic and germinal excision of *Ds*. Similarly, the transformants containing the Ac promoter fused to the transposase ORF varied three-fold in mRNA abundance and the transformant containing least mRNA showed significantly less *Ds* excision than the one containing most mRNA. This work was submitted for publication in December and we're waiting for the reviewers.

In parallel, Lluis Balcells & George Coupland have analysed three transformants containing a fusion of the soybean heatshock promoter to the *Ac* transposase. Mature plants containing this fusion and a *Ds* inserted in the streptomycin resistance gene were repeatedly heat shocked at 42°C for 2 hours. The progeny of these plants were then tested on streptomycin-containing medium. Many progeny of heat shocked plants showed variegation on their cotyledons and some were full green. The progeny of plants that were not heat shocked, showed very little or no *Ds* excision. We are now analysing this fusion in more detail.

Debbie Long, Marta Martin, Karen Ingle, June Swinburne & Kate Wilson have also been isolating large numbers of plants in which excision and re-insertion of *Ds* has occurred. This was done by crossing plants that contained the CaMV35S fusion to transposase with others harbouring a *Ds* element (marked with hygromycin resistance) inserted within a streptomycin resistance gene. The resulting hybrids were self-fertilised and the progeny selected with streptomycin and hygromycin. So far we have selected independently the progeny of 537 hybrids. In this way, we have identified at least one plant from each of 147 families that is resistant to streptomycin and hygromycin and has not inherited the transposase fusion. These plants, which probably contain a stable transposed *Ds*, will be selfed and their progeny screened for mutants. In addition, we have 155 independently isolated plants that are ➤

hygromycin and streptomycin resistant, but still contain the transposase fusion. These will be selfed and the transposase segregated from the transposed *Ds* in the next generation. We are putting a lot of effort into identifying at least 1000 of these independent transposition events in which the *Ds* has been stabilised with the aim of testing how efficient transposon tagging is in Arabidopsis.
George Coupland & group; Cambridge Laboratory, JI Centre, Norwich.

From Simon Covey...

CaMV infection of *Arabidopsis*.

We are in the progress of characterising our Arabidopsis mutant that shows an altered response to CaMV infection. We have tentatively named the mutant, DV-1 because the normal phenotype (un-infected) shows dark veins (DV) on the leaves. DV-1 is delayed in its symptom development during CaMV infection and the vein-clearing stage typical of wild-type plants seems to be absent. We hope to report on infection characters such as virus titre and minichromosome activity in DV-1 in the next newsletter. The relationship between virus titre and symptom severity in virus-infected plants is a complex one and depends upon host/virus combinations. In many systems, there are simple direct correlations between virus titre and disease severity, although this is not always the case. We have been screening genetic variants of CaMV in turnip plants and found combinations where there is not a straightforward relationship. Such combinations are of interest because they centre specifically upon virus host interactions and should help direct us towards plant genes involved in the interaction.

We also hope to report further on the genetic analysis of DV-1, including the results of backcrossings, shortly. So far, we have selfed DV-1 and found that the first generation of progeny (M_3) produce two types of phenotype: two thirds are similar to the M_2 plant in morphology and response to CaMV whilst one third are a mixture of dwarf plants with paler leaves and pale veins in which we have not been able to establish an infection. These plants also produce few flowers which do not set seed. They also need a vitamin supplement for viability. When the M_2-like population of M_3 seed are allowed to flower and set seed, the M_4 plants again segregate in two populations: one third are like the pale M_3 plants which require vitamins and the other two-thirds have dark veins, but the plants have a dwarf morphology, they flower early and we have not yet managed to infect them. We are sure there is a simple genetic explanation for this, but we poor virologists are always happy to receive additional advice.
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From Caroline Dean...

Transposon tagging.

Asphyxiated -- Anuj Bhatt, Emily Lawson & Tania Page.

We have generated 1,000 plants, each representing a germinal excision of *Ac*, from 10 different transformants. We have begun screening the progeny of these plants for putative transposon-tagged mutants. Initially, we shall screen for visible mutant phenotypes in tissue culture and in the greenhouse. Later, we plan to screen for mutants defective in response to hormones, light perception, *etc.* using different selection regimes. We are also evaluating the independence of transposition events and the inheritance of transposed *Ac* elements in some of these plants. In a few weeks we shall finish the first round of screening and should have a better idea about the frequency and the spectrum of mutations generated by *Ac*. Until then all of us are holding our breath.

"1,000 plants, each representing a germinal excision."

The *Arabidopsis* genome project.

Jo West & Renate Schmidt

Alma and Gerda have left Norwich to take up their repective Ph.D projects. Good luck to them! Jo started to work in our group in October.

Our walking efforts on chromosome 4 are still concentrating on the 10 cM region between RFLP markers, 210 and 226. Currently, three contiguous regions of 920, 830 and 400 kb lie within this region, involving 59 YAC clones. A thorough analysis of these clones has so far revealed eight chimaeric YAC clones. End-probes of these clones do not hybridise to YAC clones which should be adjacent or which span the region.

We also used the 180 and 500 bp repeat sequences which have been described by Martinez-Zapater *et al* (1986, MGG 204, 417) and Simoens *et al.* (1988, NAR 16, 6753) as probes on the EW and EG YAC libraries. These repeats represent approximately 2% of the *Arabidopsis* genome. While 10% of the EW clones showed hybridisation to these repeat sequences, only 2% of the EG clones hybridised. Both of the libraries contain approximately three genome equivalents, thus the repeat sequences are underrepresented in the EG YAC library.

A high level of YAC clones (20%) carrying the tandem repeat sequences reveal more than one YAC band when analysed using PFGE. Southern blot experiments show that all of the multiple YAC bands derived from individual clones hybridise to the repeat sequences. We believe, therefore, that YAC clones containing these tandem repeat sequences can be instable at a high frequency.

The mechanism of vernalization.

John Chandler

Following EMS-mutagenesis of a population of *fca*, individuals were selected that had lost the usual vernalization response of *fca*. Out of 280 M_2 families screened (each of 10 M_1 plants), I have now confirmed that 19 individuals (representing at least 16 independent mutations) heritably show this "loss of vernalization response" (vrn) phenotype in the M_3 generation. A further 40 putative mutants are being checked in the M_3 for heritability of the phenotype. The 19 vrn mutants have been crossed to *Ler* and *fca*, and the analysis of the F_2 of these crosses (about to be undertaken) will tell us whether the EMS-induced mutation itself causes late flowering, or whether we have achieved our aim and isolated a true mutant in the cold signal transduction pathway.

The same EMS-mutagenised population has also been screened for suppressor mutants of *fca*. From 140 M_2 families, 17 mutants flowering at the same time as Ler, and representing at least 11 independent mutations, were crossed to Ler and *fca* to characterise genetically the EMS-induced mutation. Analysis of the F_2 from these crosses is also in progress.

The range of double mutants constructed to see how the late flowering and vernalization phenotype of *fca* may be altered by a second mutation in hormone, lipid or starch metabolism, has now been extended to include *fca* with hy3, 4 and 6; axr1; aux1-7 and axr2.

An analysis of which gibberellins could accelerate flowering in *fca* showed the most effective, in decreasing order, were GA_7, GA_4, GA_9 and GA_5. These were also the most effective for Ler. This caused us to conclude that although the response is greater for *fca* than for wild type, *fca* shows no deviation from wild type with respect to a specific gibberellin.

A characterization of the photoperiodic response of Ler and *fca* is being performed, by giving plants in short days, exposure to varying numbers of long days, at different plant ages. This will show if any induction to flowering by long days for *fca* is dependent on plant age.

Genetic analysis of late flowering and vernalization response in natural isolates of *Arabidopsis thaliana*.

Jon Clarke

Fri, a locus conferring late-flowering and vernalization response, has been separated from a number of loci responsible for the late-flowering phenotype of the ecotype, Stockholm (St) (Napp-Zinn, 1957). In the background of an early-flowering ecotype, Limburg-5 (Li5), the Fri allele is seen to constitute 95% of St late-flowering character. The St allele is dominant and its late flowering phenotype is completely corrected by an eight week period of vernalization at 5°C.

A line monogenic for the St allele of Fri when crossed to the early-flowering ecotype, Landsberg *erecta* (Ler) gives a complex F_2 segregation for flowering time. Clearly, the Fri phenotype is influenced by modifying loci from Ler.

This interaction provides us with the possibility of mapping the Fri locus and, in addition, the modifying loci from Ler. Using a quantitative trait loci analysis, based upon 28 RFLP markers distributed at 20cM intervals, a population size of 50 F_3 families, and mean flowering time data, the loci will be mapped. By extending the range of environments in which the F_3 families are grown, we will also determine the influence of photoperiod and vernalization on the interaction between loci. We have flowering time data for three environments with two more in progress. Polymorphisms have been identified for 14 RFLP markers and these are being used on the F_3 families.

The St allele of Fri has now been introgressed into Ler by six backcrosses. In this background, the St allele of Fri is completely recessive, while its late-flowering phenotype remains unaffected. Its vernalization response is been examined at the moment. If the Fri locus should map in the region of an identified late-flowering mutation the introgressed line will be used to test for allelism.

During a profitable collaboration with Jeremy Harbinson at the ATO in Wageningen, we confirmed that changes in the efficiency of photosystem II were not correlated with the vernalization response, and two mutants were identifed that showed abnormal photosystem II reactions to high light at low temperatures.

C. Dean *et al*; Norwich.
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From John Doonan...

Identification and analysis of genes regulating the cell division cycle in plants.

Screening of the clontech λgt11 cDNA library identified four clones that hybridised to the Aspergillus bimG gene. Sequence analysis reveals one of these clones to have the potential to encode a polypeptide very similar to the bimG phosphatase. Complementation testing is underway to determine if it has similar function. Probing a genomic Southern of Arabipodsis reveals that the gene is present at one copy/haploid genome.

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From David Evans...

Molecular biology of the regulation of the plasma membrane calcium transporter in *Arabidopsis* and Zea.

On the biochemical front, work is progressing well with studies on the purification, reconstitution and membrane biology of plant calmodulin-stimulated ATPases. Recent work by Per Askerlund has addressed the question of whether there is more than one isoform of the calmodulin-stimulated calcium pump, located in different membranes (*i.e.*, not just the plasma membrane) and showing different properties and regulation. This work is important because it addresses an as yet unresolved question concerning the origins and homogeneity of material used for reconstitution studies and also questions concerning the rôle of calmodulin-stimulated Ca transport *in vivo*. Reconstitution studies using highly purified calmodulin-stimulated ATPase and using oxalate entrapment give excellent specific activities.

David Coates reports from Leeds that in view of the low-abundance of the Ca pump message, he has adopted a redesigned PCR approach. RNA was isolated from young Arabidopsis seedlings grown in the dark in liquid culture. Total RNA was reverse transcribed and the single-stranded DNA used for PCR amplification with carefully designed primers. The crude result was a range of fragments from 500-1000 bp. These were subcloned into BlueScript and the inserts characterised by size and preliminary sequence. Of 36 independent clones, eight have so far been sequenced. Of these, seven have different sequences, some of which can be identified by homology with sequences in the GenBank data base. One shows similarity to cucumber glycerate dehydrogenase, a second to GTP-binding proteins, the others are as yet unidentified. Of the remainder, ➤

one contains ATPase-like motifs and further characterisation of this clone is in progress. The other unknowns are also being investigated. It is clear that PCR amplification of plant material using redundant primers is beset by problems. Not least of these is the presence of large amounts of chloroplast DNA and RNA, which act as substrate for amplification - we have cloned at least two chloroplast derived sequences so far. Life goes on....

In Oxford, Joy Boyce has obtained a new range of antibody and plasmid probes to various isoforms of mammalian calcium pumps and is currently screening them. These seem to be our best chance yet; if they fail, it won't be for want of a rigorous approach to all the available methods of cloning this gene and will indicate that we are onto an extremely difficult problem, which will take a lot of effort to resolve. Workers who cloned the mammalian Ca pumps cheerfully inform us that they, too, had a huge amount of hassle -- not least because of the extremely low abundance of the message. Our plea in the previous newsletter (*A-rap-a-bop-sis*) for a 'really good cDNA library enriched in membrane protein sequence' produced nothing, but a similar message on the bulletin board has yielded two libraries, both of which are central to our new screening. *(Non-bulletin-board subscribers, please take note -- ACM.)*

Work on the other Ca binding protein (calreticulin-like) homologue identified is going well. Anti-calreticulin antibodies recognise plant membrane-associated proteins and clones detected by this antibody from a plant cDNA library are about to be sequenced. This continues to reinforce our suggestion that plants have a calreticulin-like protein (which our work shows is not calsequestrin, also identified in plants) and which may be a central protein in calcium signalling cascades. Such proteins are essential to the function of calcium pumps in intracellular stores.

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From Gary Foster...

Regulation of *Ds* transposition in higher plants and evaluation of rapid techniques for the cloning of flanking DNA.

Work is going very well, but due to the stage we are now at, we have very little to report in this newsletter -- even though all is going well. The reason is that we have built all our required constructs and are now sitting waiting for our plants containing our constructs to flower so that we can cross them. To date, we have engineered the transposase required for the transposition of the *Ds* transposable element behind a microspore specific promoter into tobacco and Arabidopsis. This promoter should express transposase in microspores thus giving high levels of germinal excision events and will not express in vegatative tissues, which might give rise to somatic transposition. When these plants are crossed to a plant containing a *Ds* element and the seeds collected, grown, and selfed; they should give high percentages of progeny with a stable germinal excision of *Ds*. As the seed grows up into a proper plant, the *Ds* element will not move at any stage, until microspores are again formed. The promoter is switched on again, producing transposase and allowing *Ds* to move again, and so on and so on. This high level of germinal excision is a goal wanted by all "transposase taggers."

We will therefore report on the success (or failure) of this approach in the next newsletter. Until then, all transposon taggers everwhere keep your fingers crossed.

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From Ian Furner...

Towards a molecular genetics of apical development in *Arabidopsis thaliana*.

The fate maps of the L_2 layer of the seed and inflorescence meristems have been finished and submitted for publication. These studies using the *alb 1* mutation and X-ray induced sectors suggest there are relatively few restrictions on cell fate in the Arabidopsis apex. As in other plants, cells of L_2 are recruited in a lineage independent-position dependent way to form the organs of the mature plant. Arabidopsis has distinct L_1, L_2 and L_3 layers in the meristem set up during embryogenesis and these represent distinct lineages. However, occasional sectors occur that appear to cross between these layers.

"...a short plastochron mutant which produces leaves in the normal phyllotaxy three times as fast."

The *alb1* mutation has been crossed into four lines which affect apical development with the aim of studying cell fate in the mutant meristems. The lines are: *fca* (late flowering), *hy2* (early flowering), *clv1* (enlarged apex) and *lfy1* (flowers replaced by leaves and structures intermediate between flowers and shoots). We hope to gain some insight into how these mutations change cell fate and meristem function. We are starting some studies of cell autonomy by using developmental mutants and linked colour markers. This approach involves producing marked mutant sectors in heterozygous plants using X-rays. This method is limited by the availability of colour mutants linked to the gene of interest. We have been collecting new albino, yellow and pale mutants with the long term aim of mapping them and increasing the number of developmental mutants that can be studied using this method.

The screen for EMS induced mutants affecting the apex had yielded several interesting lines. One of the more interesting ones is a short plastochron mutant which produces leaves in the normal phyllotaxy three times as fast as wild-type. We have two recessive alleles of this locus and have started mapping it. Another interesting group of mutants (three independent isolates allelism not yet tested) are early flowering. This recessive phenotype is associated with a great reduction in leaf size and the plants are usually infertile. These plants do not have the etiolated appearance of the early flowering *hy2* line. Several mutants disturbing apical

meristem development have been isolated as segregating heterozygotes and are too abnormal to flower as homozygotes. These range from mutants which show no shoot development to others in which the apex overgrows to form callus and development stops. We are continuing this screen and extending it to X-ray mutagenised families.

Paul Davison the postdoc involved in the c-DNA work of this project has left and we intend to emphasise the genetic/fate mapping work for the rest of the project.

Ian Furner & Joanne Pumfrey; Department of Genetics, University of Cambridge.

From Nic Harberd...

Genetics and molecular biology of growth and development in *Arabidopsis thaliana.*

Good progress is being made. We welcome post-doc Pierre Carol too.

Nicholas Harberd & group; J.I. Centre, Norwich, UK.

(Following on from his previous Clarissa-like epic, Nic has reverted to the seventeen syllable, Haiku. He promises another saga for the next edition -- ACM.)

From Nick Harris & Phil Gates...

Development of the silique of *Arabidopsis.*

Our work has continued to follow both cytological/cytochemical and molecular approaches in analysing the development of the fruit of Arabidopsis. You may recall that the initial phase of development, which establishes the basic patterns of the ovary walls and ovules, is switched into a second phase by fertilisation. The second phase of ovary wall development can be triggered by applied GA to give parthenocarpic fruit in which there are no 'complicating' hormonal influences from the developing embryos and seeds.

On the structural side, Jackie has been looking at the patterns of key enzyme activities associated with the differentiation of particular cell types and layers which are linked to the establishment of both the abscission zones and the physical properties of the fruit which lead to it being shatterable. She has also been comparing the development of several silique mutants, which have come from our screening of EMS-treated seed. The cytochemical work has included *in situ* hybridisation, immunocytochemistry and assay of functional enzyme activities. The last has sometimes been a little 'fiddly' as it requires cryo-sectioning of isolated individual, and rather small, early ovaries. However, results of, for example, esterase activity in pre- and immediately post- fertilised ovaries look particularly interesting. This is especially so when low levels of activity are 'enhanced' by image processing to reveal some intriguing patterns.

On the molecular side, Lesley Edwards has made several cDNA libraries and is currently involved in analysing the products of a subtractive screen of an early silique library with one from mature leaves. The silique contains a prominent photosynthetic mesocarp, but our cytological studies suggest that the more interesting 'action' is associated with the development of the exocarp, septum and, particularly, the endocarp. To remove the photosynthetic and housekeeping activities from the early silique library, its antisense cDNA was hybridised with a 10 fold excess of biotinylated sense, leaf-derived cRNA. Hybrids were removed using streptavidin paramagnetic particles. Second strand synthesis was carried out on the residual single-stranded cDNAs, and EcoRI/XbaI digests were ligated with pGEM2Z and transformed into JM109. The Eco sites disappeared (?!) so blue white screening with Xgal/IPGT, and nuclease digests were used to confirm inserts. A group of these are now being characterised. The SP6/T7 promoters serve as primary sites for dideoxy sequencing, and two putative silique-specific clones are being sequenced. Screening of EMS-induced mutants continues.

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From Pat Heslop-Harrison...

Localisation and characterisation of tandemly repeated DNA sequences in *Arabidopsis.*

We have been using labelled DNA from long, mapped clones, as *in situ* probes on metaphase chromosomes and interphase nuclei. Our results show hybridization sites as brightly fluorescent dots on a dark background, and we see two dots when we use one probe, and four when we use two simultaneously, on each interphase nucleus. With the probes p210 and p240 we find two big dots and two little dots in each interphase.

The same chromosome preparation does not always show clear hybridization signals on both metaphase chromosomes and interphase chromatin. This implies that there are differences in the denaturation conditions needed for interphase and metaphase chromosomes. It seems that higher temperatures or longer times or both are required to see signal at interphase rather than metaphase. (Yes -- it is the other way round from what you might think!) We also speculate about differences in the conditions required to denature active versus inactive chromosome segments.

Results from mapping of human chromosome 9 by Barbara Trask and friends at Livermore lab. in California show that the *in situ* hybridization method to interphase nuclei can define new linkages between their long contigs. Her work shows that the interphase mapping approach is viable, although it relies on the chromosome arms decondensing linearly from metaphase to interphase. We suspect the same holds true in Arabidopsis, but need to show this by using three linked probes and finding them always in the same order -- the one labelled with biotin and the other two with digoxigenin.

Jola has returned to Vienna, but we are continuing our close collaboration on the molecular cytogenetics of Arabidopsis.

Jola Maluszynska & J.S. (Pat) Heslop-Harrison, Karyobiology Group, JI Centre, Colney Lane, Norwich, NR4 7UH, U.K.

From Eric Holub...

Identification and mapping of genes for resistance to fungal pathogens of *Arabidopsis*.

The hypothetical model we presented in the previous newsletter provides a framework for genetic analyses and highlights the complexity of the interaction between *A. thaliana* and *P. parasitica*.

> "...the complexity of the interaction between *A. thaliana* and *P. parasitica*."

Validation of the model continues with analysis of F_2 populations from the half-diallel cross. Nearly all F_2 populations from crosses made between the parents; Wein, Nd0, Oy0, Col0, La-er, RLD, Tsu0, and Kes37 have now been tested against isolates CALA and EMOY2. The data appear to be consistent with the model although some progeny testing will be needed. For this purpose, seed is being produced from selected F_2 plants. F_2 progeny sizes will be increased for several crosses particularly those where both parents are resistant. This analysis has provided us with four putative genetic loci to investigate in more detail and further work with the diallel cross will undoubtedly reveal new genes for investigation.

In essence, the hypothetical model is like an imperfect chart of a vast ocean, and accurate description of its features is an enormous challenge. For smooth sailing in the ocean, we have chosen a suitable vessel and set our bearing. The course will be to investigate what appear to be four different classes of phenotypic reaction, all of which are easy to score using living material with or without the aid of a dissecting microscope. The interaction phenotypes include: full susceptibility with sporophores profuse and visible 3 days after inoculation (dai); delayed sporulation with sporophores absent 3 dai, but visible and usually sparse 7 dai; no sporophores produced, but flecks of necrotic host cells visible to the naked eye 7 dai; and no sporophores produced, but necrotic "pits" visible to the naked eye at 3 dai. The pits are discrete epidermal lesions up to 1mm in diameter by 3 dai and are often surrounded by a chlorotic halo. They have usually coalesced four days later, but a seedling responding in this way will continue to grow even though the cotyledons are entirely necrotic. Our vessel will be the cross Col0 x Nd0 in which all four interaction phenotypes are seen among F_2 progeny following inoculation with the isolates CALA and EMOY2.

In response to CALA, the fully susceptible reaction of Nd0 segregates in the cross together with the flecking phenotype of Col0. The flecking reaction is probably controlled by an incompletely dominant allele at a single locus. In response to EMOY2, the delayed sporulation reaction of Col0 segregates in the cross together with the pitting phenotype of Nd0. All four phenotypes can be readily scored in the F_3 generation so we anticipate being able to determine the genotype at both loci (see Table). We believe that a single allele controls each response of "flecking" to CALA, and "pitting" and delayed sporulation to EMOY2. The pitting allele (allele A) and flecking allele (allele B) are now our prime targets for molecular analysis (see report by Jim Beynon). It should also be possible to map the locus controlling delayed sporulation of Col0 (allele D) following inoculation with EMOY2, but the phenotype seems to be more sensitive to differences in the physical environment, inoculum concentration, and/or modifier genes (see Table; compare F_3 segregations from F_2 plants with the *aaDd* putative genotype).

Thirteen *A. thaliana* accessions have been found on which sporulation by *A. candida* does not occur or is delayed following inoculation with an isolate collected from East Malling (EMAL). Nine of these putatively resistant accessions were collected from locations in the UK and four from Germany. The responses include: no pustules and no macroscopically visible necrosis; necrotic flecking without pustules; discrete chlorotic patches without pustules; and delayed, sparse production of pustules. Crosses between plants selected from these accessions are being made by Edemar Brose from Jim Beynon's lab. These crosses will be used to identify new genes for resistance to *A. candida* isolate EMAL.

Table. Representative sample of Col0 x Nd0 F_3 families showing segregations for response to P. parasitica isolate EMOY2 and predicted genotype of each F_2 parent.

F_3 family	F_3 segregation[1]	Putative F_2 Genotype[2]
911448	0:0:0:10	*aadd*
911451	0:3:9:3	*aaDd*
911455	0:2:8:0	*aaDD*
911443	0:0:8:2	*aaDd*
911446	0:6:2:2	*aaDd*
911442	0:5:5:0	*aaDD*
911430	14:0:0:0	*AA__*
911469	10:2:0:0	*AaDD*
911470	7:5:1:2	*AaDd*
911436	12:0:0:7	*Aadd*

[1] Data (left to right) are numbers of plants in each of four phenotypic classes: pitting necrosis of cotyledons 3 days after inoculation (dai), no sporophores; necrotic flecks 7 dai, no sporophores; sporophores absent 3 dai but present 7 dai; sporophores present 3 dai and sporulation profuse 7 dai.

[2] Predictions for the status of two putative loci for resistance to EMOY2. *A* is a completely dominant allele from the Nd0 parent causing pitting necrosis; and *D* is an incompletely dominant gene from the Col0 parent causing delayed asexual sporulation. The genes appear to segregate independently.

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From Gareth Jenkins...

Isolation and characterisation of photoregulatory signal transduction mutants in *Arabidopsis*.

In the last Newsletter we reported the isolation of several new mutants altered in blue light responses. Our recent effort has been to characterise these as far as possible. In addition, we have continued to study the existing blue light response mutant *hy4*, particularly with regard to gene expression. Although the results are only preliminary at this stage, *hy4*

seems to merit further study. This latter work was carried out by a German undergraduate, Antje Ziemann, who visited us, self-financed, for five months. Antje became addicted to northern blots and hopes to return to continue work for her diploma thesis (and to climb a few more mountains). We would very much like her to come back, but finding money for this purpose is very difficult. Hence we would be delighted to hear from any potential benefactors who would like to sponsor Antje with a bursary!

Some of our new mutants were isolated by screening M_2 populations under blue light for a long-hypocotyl phenotype. In order to define the phenotypes, we have been analysing several parameters of development that are regulated, at least in part, by blue light. We initially measured; cotyledon expansion, leaf expansion, anthocyanin content, and chlorophyll content. We recently started studies of gene expression. We have also crossed the mutants with each other and with *hy4* to check for allelism. The results are complicated by incomplete dominance of the long-hypocotyl phenotype, but we are convinced that the genetic analysis supports our conclusion from physiological studies that we have several new mutants which are different from each other and from *hy4*.

"We have isolated several putative mutants by screening M2 seed of transgenic plants."

Readers may remember that one of our main objectives was to isolate new photoregulatory mutants by screening mutagenised seed of various transgenic populations. Although arduous, this approach has proved successful. We have isolated several putative mutants by screening M_2 seed of transgenic plants and these mutants are being characterised. A new graduate student, Morgan Shaw, supported by an AFRC studentship has joined the project and is involved in this work.

Gareth Jenkins, Karen Deeney, Jennie Jackson, Morgan Shaw & Antje Ziemann; Departments of Biochemistry and Botany, University of Glasgow.

From Kerrie Jones...

Ammonium toxicity in *Arabidopsis*.

Our original approach to cloning the Arabidopsis gdh gene(s) was nested PCR using redundant primers designed from a GDH sequence alignment. So far, this method has not born fruit and we have decided that specific primers may be required. Purification of the Arabidopsis GDH is therefore underway. We should have pure protein very shortly from which we can obtain peptide sequence enabling us to design specific primers. We will also raise antibodies to the protein which can be used to screen the cDNA library. Construction of transgenic plants carrying the *E.coli* gdhA gene is progressing steadily.

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From Peter Jordan...

The genes encoding the early enzymes of the chlorophyll biosynthesis pathway in *Arabidopsis thaliana* and their regulation.

Molecular biology

Work is continuing to complete the sequencing of the *hemL* cDNA from the PCR generated segment previously obtained. Screening is also in progress using the DNA fragment with the genomic library of *A. thaliana*. Results of the screening both by PCR and by ^{32}P hybridization to nylon membranes are expected shortly. No obvious success with the search for *hemB*, *hemC* and *hemE* cDNA or genes have yet been obtained, despite several apparently positive indications, and this work is continuing.

Isolation of enzymes

Comparisons have been made between the activity of the early enzymes of chlorophyll synthesis (glutamate 1-semialdehyde transaminase, porphobilinogen synthase, porphobilinogen deaminase and uroporphyrinogen decarboxylase) in several species of plants. Spinach, lettuce, cabbage and *A. thaliana* have all been studied. Arabidopsis leaves are quite suitable for the isolation of enzymes. The only disadvantage is the obvious one of its small size and the difficulty in growing large numbers of plants.

After initial ultrafiltration of plant tissue homogenates by ultracentrifugation, ammonium sulphate fractionation and ion exchange chromatography, porphobilinogen deaminase has been purified by heat treatment and fplc. The units of enzyme are quite low and the purification is being scaled up. Uroporphyrinogen decarboxylase has been partially purified using fplc. This enzyme appears to be sensitive to low pH. Some evidence of proteolytic digestion is apparent and current studies involve the use of PMSF and benzamidine. Uroporphyrinogen decarboxylase is stimulated by 2-mercaptoethanol. Both enzymes are unstable at early stages of purification.

P.M. Jordan; Biochemistry & Molecular Biology Laboratory, Queen Mary & Westfield College, University of London, Mile End Road, E1 4NS.

From Keith Lindsey...

Insertional mutagenesis in *Arabidopsis thaliana*.

The aim of this work is to develop a gene tagging system based on the activation of the *gus* reporter gene by native gene regulatory sequences. We have used our modified version of the Valvekens root-explant system to generate about 2100 *Arabidopsis* transformants containing a promoterless *gus* gene. We have screened about 400-500 of these lines, both for GUS activation in diverse organs and tissues, and for aberrant phenotypes, and are studying genetic segregation and transmission. As we indicated in the last newsletter, the range of *gus* expression patterns is enormous, and in this last third of the project we are directing our efforts to a molecular analysis of only a few lines. This is involving a detailed analysis of T-DNA organisation, and characterisation of *gus* expression during the plants' life cycles, for lines expressing specifically or predominantly in (a) the tapetum, (b) the stigma surface, (3) mature pollen, (4) vascular tissue and (5) zygotic embryos. We have had some ➳

success with the use of inverse PCR to amplify genomic sequences that flank the T-DNAs, but in some cases there seems a lack of useful restriction sites available and we are making partial genomic libraries to clone out tagged sequences in specific lines.

There have been one or two useful spin-offs of this work. One includes the use of lines that express in the pollen, in a MAFF-funded project in collaboration with David Twell here, to study the environmental impact of transgenic plants. The pollen 'genetic markers' potentially provides a means of identifying transgenic pollen in a mixed population, and of studying environmental factors that may affect gene expression in pollen.

"The transparent testa and male sterile phenotypes, at least, are linked to T-DNA sequences."

We have also identified a number of segregating aberrant phenotypes in the T_2 and T_3 generations. These include: transparent testa, dwarf, curly root, short root, agravitropic root, bushy shoot, embryo-lethal, albino, triple cotyledon, cotyledonless and rootless. Interestingly, a line which expresses *gus* in the tapetum (apparently specifically) is male sterile in the homozygous condition. Artificial male sterility in one fell swoop? Efforts are currently underway to determine which of these phenotypes are linked to the T-DNA inserts, by co-segregation analysis; linkage would greatly facilitate the isolation of the mutant genes. Preliminary results indicate that the transparent testa and male sterile phenotypes, at least, are linked to T-DNA sequences.

We have found that the amount of work to do on this project has increased exponentially since we got the transformation system working so efficiently, but by focussing on specific developmental processes we are hopeful and excited about the future results this approach, and this resource of transformants, will generate.

Keith Lindsey, Mike Clarke, Jennifer Topping, & Wenbin Wei; Leicester Biocentre, University of Leicester.
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From Andy Maule...

Identification and exploitation of the interaction between a protein and host façors which control virus spread.

Significantly, surprisingly and encouragingly, we have found that a large in-frame deletion in the movement protein gene of CaMV can be complemented *in vivo* by co-infection with wild type virus. The experiment, based upon co-agroinfection of the mutant and wild type virus into the host plant, results in accumulation throughout the plant of approximately equal amounts of the two viral genomes without any recombinational rearrangement of the mutant. Why is this significant? It tells us that the P1 protein does not have an additional cis-acting function related to virus replication or encapsidation and hence, that future experiments with other mutants are likely to be free of these complications. It also tells us that the P1 function itself can act in trans. Interestingly, an out-of-frame mutant was not complemented in a similar experiment, a result which probably relates to the complex modes of expression of the downstream cistrons on the CaMV 35S RNA.

"A large in-frame deletion in the movement protein gene of CaMV can be complemented *in vivo*."

In the light of the above observations, it was distressing to find that our P1-transgenic *Arabidopsis* did not complement the same virus mutant, although our inability to detect the protein product in these plants, despite the presence of CaMV gene I-specific RNA, probably provides the explanation. We can only assume that the addition of small 5' and 3' noncoding sequences to the transformation vector in some way reduced the efficiency of expression. We have started a further round of transformation after precisely replacing a GUS insertion with the gene I sequence.

Three other strategies are now available to us for the testing of mutants. First is the assay *in vitro* for P1 single-stranded nucleic acid binding activity (V. Citovsky, UC Berkeley). Carole is currently visiting the Berkeley lab. to confirm their observations with some of our early clones used for the P1 expression in insect cells from baculovirus vectors. Second, is the leaf disc assay described in an earlier newsletter and third, is a protoplast system where the requirement for cell-to-cell spread of the virus is eliminated. The last is the most problematic since in contrast to most other uses of protoplasts, we need a system where a large proportion of the population (*e.g.*, 30%) survives for at least 72h and show an infection when inoculated with multimeric cloned CaMV DNA constructs. If anyone has new protocols that lead to transient expression or transformation with this sort of efficiency we would be happy to hear from them. The construction and analysis of mutants will now start in earnest (wherever that might be).

The characterisation of the *Arabidopsis* protein that binds E.coli-expressed P1 is continuing. Although our biggest hopes were gone when we found that P1 also binds non-specifically (?) to all λZAP plaques from an *Arabidopsis* cDNA library.

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From Keith Mitchelson...

Identification and cloning of hypervariable loci from *Arabidopsis thaliana*.

Since our last report we have continued to screen an *Arabidopsis* library in λ (from Denis Murphy) with exogenous probes which have demonstrated hypervariable difference signals between *Arabidopsis* ecotypes. Putative positive λ clones have been identified and are now being purified through several round of selection. We have observed that phage plaques produced by clones carrying some repeat positive sequences are extremely small. Since the instability of repeat sequences from other organisms has been known to reduce stability of recombinant molecules in *E. coli*, a range of different recombination-minus *E. coli* hosts are being tested. Sub-cloning λ DNA fragments into plasmids and identification of endogenous *Arabidopsis* elements which hybridise to the

probes is the next step. This will be followed by re-probing of *Arabidopsis* genomic DNA digests with endogenous probes to identify informative alleles.

Andy and Charlotte Shui (an honours student) have also continued screening of YAC clones (EG-Columbia library) with the M13 probe. Putative positive clones are present in YAC chromosomes with each 96-well plate yielding 2-3 strongly hybridising clones and 2-4 less strongly interacting clones. Screening of individual YAC clones has confirmed the presence of both strong and weak hybridising elements, consistent with the picture seen with pooled YAC clones. Several of the stronger hybridising YAC clones are being isolated on LM-agarose in order to show co-identity with *Arabidopsis* genomic DNA fragments which hybridise to M13 probe.

Andy Porter & Keith Mitchelson; Dept. of Molecular & Cell Biology, University of Aberdeen.

From Steven Neill...

Identification of water stresses and ABA regulated genes using wilty mutants of *Arabidopsis thaliana*.

We've made progress on several fronts since the last edition of the Newsletter. As the main thrust of our work is to investigate the effects that drought stress and abscisic acid (ABA) have on gene expression, it's clearly important that we get reliable data on ABA levels in different tissues subjected to various treatments. We routinely measure ABA using a radioimmunoassay based on the anti-ABA monoclonal antibody MAC 62, kindly supplied by Steve Quarrie, Cambridge Laboratory, Norwich. To confirm the veracity of our data, we have validated the RIA for *Arabidopsis* tissues using GC-MS in collaboration with John Lenton & Steve Croker from Long Ashton Research Station.

We've also spent a lot of time and effort on making a subtracted cDNA library enriched for stress-induced sequences; although we have progressed, we're not there yet. Our first approach has been to use cDNA-cDNA hybridisation. Mike Bulman (supported by SERC) has used PCR successfully to generate large amounts of cDNA, which do appear to represent "real" plant cDNA as we get good signals when they are probed with an *Arabidopsis* RUBISCO probe or with A1494, a stress-inducible gene (see below). Using photobiotinylated cDNA and avidin-coated beads, Mike has been

> "...gene which appears to be induced by drought or ABA."

successful at removing unwanted hybrids from the subtractive hybridisation mix and generated cDNA from the enriched residue. This cDNA preparation didn't give much of a signal when probed with RUBISCO, which was good, but didn't give a signal with A1494 either, which wasn't so good! Consequently, Mike is repeating these hybridisations, but using biotinylated mRNA instead of cDNA to remove common sequences.

We've also progressed in our studies of drought and ABA regulated gene expression and, in collaboration with Andy Phillips, another Long Ashton scientist, Jackie has identified a gene which appears to be induced by drought *or* ABA. This gene, presently termed A1494, has a mRNA transcript size of 1250bp and is expressed at only low levels in turgid shoots. Rapidly-imposed drought stress (in the form of a 10% loss in fresh weight) induces a rapid increase in the level of its mRNA (as determined by northern analyses). There is a detectable increase within 30 min which continues up to 6h, after when the levels decline again. This drought stress also results in a rapid and substantial increase in the endogenous ABA concentration from *c.* 20 ngg^{-1} fwt to 80 ngg^{-1} fwt by 6h and 120 ngg^{-1} fwt by 8h. ABA treatment of shoots also results in an increase in the amount of A1494 mRNA, again the effect being greater after 4h compared to 8h. However, it would appear that the increased expression of the A1494 gene is not due solely to an increased endogenous ABA content resulting from the drought stress, as its expression is also induced by drought stress in the ABA-deficient *aba*^{1} mutant. A1494 mRNA is not detectable in turgid *aba*^{1} shoots, but in response to stress its levels increase substantially. The ABA content on the other hand does not increase, remaining at *c.* 10 ngg^{-1} fwt. ABA treatment also induces A1494 expression in *aba*^{1} shoots. We are about to look at A1494 expression in the ABA-insensitive *abi* genotypes. Furthermore, Andy has got a partial sequence for the pA1494 insert and found some homology to 15a, a stress-inducible gene previously isolated from pea (Guerrero, Jones & Mullet, 1990 *Plant Mol. Biol.* 15, 11-26)

In parallel with these experiments we've also found that drought stress leads to a substantial reduction in the levels of RUBISCO transcripts in *Arabidopsis* (determined by northern analyses using an *Arabidopsis* RUBISCO probe supplied by Dr. E. Krebbers, Plant Genetic Systems, Gent, Belgium). ABA treatment also down-regulated RUBISCO mRNA levels.

Mike Bulman, Steve Neill & Jackie Williams; Bristol Polytechnic.

From Helen North...

Cell cycle control genes in *Arabidopsis*.

Attempts at screening our meristematic cauliflower λZAP library with a highly conserved region of the *S. pombe* cdc25 gene have so far been unsuccessful. In order to determine how many plaques we should expect to screen, we are now using a human α-actin gene, which due to the high homology between actin species, cross hybridises on northerns to *S. pombe* and cauliflower transcripts. As we are hoping to complement *S. pombe* cytokinesis mutants, which have been shown by immunofluorescene staining not to proceed with expected actin movements, it may be possible to complement the function of some of these with any plant actin clones we identify.

From test transformations of the *S. pombe* cdc7 cytokinesis mutant,we now have restriction maps of three different classes of rescuing plasmid, and possibly have more to come

Helen North & Jeremy Hyams; Dept. of Biology, UCL.

From Jane Parker...

Infection of *Arabidopsis thaliana* with *Xanthomonas campestris* pathovar *campestris*: The search for resistance genes.

The cosmid clone, pIJ3130 contains DNA from *Xanthomonas campestris* pathovar *raphani* strain 1067 which, when transconjugated into the virulent *X. c. campestris* strain 8004, renders it avirulent towards *A. thaliana* ecotype Col-0 and most other ecotypes tested. It does not, however, affect the virulence of strain 8004 on several different *Brassica* species, and is therefore specific in its action.

We have spent some time defining the region of avirulence activity by a combination of subcloning and Tn*5*-transposon mutagenesis. This has revealed a stretch of approximately 2kb which contains the 'avirulence' gene. A transposon insertion within the gene causes reversion to a virulent phenotype by the criterion of disease symptom development and by growth of the bacteria in Col-0 plants.

Chris sequenced the DNA fragment and found an open reading frame encoding a protein of 66.6kDa. A detailed analysis has revealed a perfect '*hrp*' box approximately 30bp upstream from the putative translational start site. This is a consensus sequence which has been identified in the 5' untranslated region of some *Pseudomonas syringae* avirulence genes as well in several genes contained within the so called '*hrp*' cluster. The activity of which is essential for pathogenicity on a host plant and for the formation of a hypersensitive resistance response on a non-host plant. The biochemical function of these genes is not yet understood, but we can speculate how they might interact with host-range determinants such as the avirulence genes in the plant-pathogen interaction.

Another interesting feature of the *X.c. raphani* avirulence gene is a stretch of 14 hydrophobic amino acids in the N-terminal region of the protein which is preceded by several basic residues and is followed by a bacterial consensus cleavage site. This suggests that the protein is targeted at least to the periplasmic membrane and possibly exported outside the bacterial cell.

We have come to bit of a dead end in our search for a *A. thaliana* ecotype that is sensitive to *Xcc* 8004(*avr*Xca) as a starting point for the genetic analysis of resistance in Col-0 plants. Our only candidate, Kas-1, has been erratic in its response, and is generally less susceptible to *Xcc* 8004(*avr*Xca) than to *Xcc* 8004 alone. We are testing Kas-1 again together with several other ecotypes, but I suspect the susceptible reaction is not distinct and reproducible enough to use it in a segregation analysis.

It has been noted in a previous report that the presence of *avr*Xca in Xcc 8004 attenuates growth of the bacteria '*in planta*' at low inoculum concentrations, but does not cause the formation of a hypersensitive response at high inoculum doses. Instead, there is a delay and reduction in disease symptom expression. This type of resistance reaction has not been studied in detail and it is of interest to determine what plant processes, for example changes in gene expression and biochemical signalling mechanisms, distinguish it from the rapid plant-cell necrosis characteristic of the hypersensitive reaction.

Jane Parker, Christine Barber & Michael Daniels; The Sainsbury Laboratory, John Innes Centre, Norwich.

From Kevin Pyke...

An analysis of leaf development and chloroplast division in *Arabidopsis thaliana*.

We have started to analyse specific aspects of the mutant phenotype of two of the most extreme arc mutants with altered chloroplast accumulation; arc1, with 30% more small chloroplasts, and arc3 with a very few large chloroplasts compared with wild type.

(i) The levels of chloroplast (&) DNA in these mutants were assessed by probing thin cut sections (7 μm) of first-leaves fixed and embedded in polyethylene glycol (PEG 1500) with the DNA specific fluorochrome 4'6-diamidino-2-phenylindole (DAPI). RUBISCO has been quantified using a fluorescent-tagged antibody.

(ii) The cell-specific nature of the mutant phenotypes of arc1 and arc3 is being investigated by analysing chloroplasts in epidermal cells in these lines after iodine staining of epidermal peels. Whilst chloroplast numbers are similar in both mutants and wild type (at around eight per epidermal cell), significant variation in chloroplast size is observed in the mutant arc3 with a 50% increase in epidermal chloroplast size compared with wild type.

(iii) We have also started a mapping study of arc1, arc2 and arc3 by crossing onto the multitester line, W100 to determine the chromosomal location of these mutant loci.

A paper is in press in Plant Physiology entitled, "Chloroplast division and expansion is radically altered by nuclear mutations in *Arabidopsis thaliana*".

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From Peter Quinn...

Thermal tolerance of fatty acid desaturase mutants of *Arabidopsis*.

Good progress can be reported on the isolation of single molecular species of galactolipids and their characterisation by dynamic X-ray diffraction methods. We have now prepared pure distearoyl derivatives of monogalactosyl and digalactosyl diglycerides using semisynthetic methods. Changes in structural parameters have been recorded in aqueous dispersions of these two lipids during temperature scans identical to those performed by differential scanning calorimetry. Analysis of the diffraction data is almost complete and at this stage it is clear that a comprehensive thermodynamic picture of the phase behaviour of these lipids will be resolved. This will set the scene for the isolation and characterisation of the unique molecular species of galactolipids in *fad* mutants of Arabidopsis and our efforts will be focussed on this over the coming months. In tandem with this work we intend to initiate our freeze fracture studies of the structure of the thylakoid membrane of fad mutants.

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From Christine Raines...

Genetic analysis of regulatory factors determining the development of the photosynthetic apparatus of plants.

Please see the sad report about Philip Horsnell in the News section of this Newsletter -- ACM.

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From Colin Robinson...

Isolation and analysis of Arabidopsis chloroplast biogenesis mutants.

The screening protocol is aimed at identifying mutants that are defective in thylakoid protein transport/ maturation. M_2 seedlings which are able to germinate, but not photosynthesise, are harvested and leaf extracts western-blotted to detect accumulated intermediate forms of thylakoid lumen proteins. Using this procedure, about one thousand seedlings have been screened and two putative mutants isolated. In both cases, the lumenal 33kDa photosystem II protein is clearly present as the intermediate-sized form. This is encouraging. We are unable to determine the locations of the intermediate forms from these preliminary findings. The polypeptides could be present in the stroma (if the mutation is in the thylakoid import machinery) or in the thylakoid lumen (if the thylakoid processing peptidase is inactive). However, either type of mutant is of interest. The major task now is to have to return to the parental mutant M_1 seed groups and detect the mutations once more, to be able to propagate the mutation in the heterozygote form. These plants should set seed in a few weeks' time, and hopefully (fingers crossed) a quarter of the progeny will be homozygous mutants. Meanwhile, we are completing screening of the mutant seed groups that were collected.

Colin Robinson & Alison Creighton; Dept. of Biological Sciences, University of Warwick.

From Steve Slocombe...

Temporal regulation of the stearoyl-ACP desaturase during embryogenesis.

Evidence indicates that in the developing *B.napus* embryo, the oil synthesis enzyme stearoyl-ACP desaturase is expressed much earlier than oleosin, which encapsulates oil bodies. The desaturase transcript peaks at 45 days post-anthesis (DPA) whilst that for the oleosin does not do so until 70 DPA. This is consistent with the observation that oleosin accumulates after oil droplets appear. A *B.napus* genomic DNA clone of the desaturase has been derived and the promoter region sequenced. Promoter-gus constructs will be transformed into tobacco to analyse the expression of the desaturase gene. Microspore embryo cultures represent a useful system to examine potential effectors such as ABA and jasmonic acid *in vitro*. This will be used to determine the factors involved in the expression of desaturase in relation to oleosin.

Steve Slocombe & Denis Murphy; John Innes Centre, Norwich.

From Alison Smith...

Investigation of the gene for hydroxymethylbilane synthase from *Arabidopsis* in transgenic tobacco plants.

Huguette Albrecht has now left the project and a research student, Saw Hoon Lim has taken over her work. Within a month, Saw Hoon has successfully identified one, and possibly two, genomic clones for HMBS from our Arabidopsis library. She has subcloned a number of fragments from one of the clones, and identified a region which corresponds to the middle of the coding sequence for HMBS. She is now about to walk up the clone to find the 5' end of the gene, and thus, hopefully, the promoter. As soon as this is available, we will be in a position to prepare chimeric constructs with GUS, to study the tissue specificity of the HMBS gene. Meanwhile, Mike Witty is in the process of identifying a tobacco cDNA clone for HMBS so that we can prepare antisense constructs, under the control of a tetracycline-inducible promoter, for transformation into a tobacco line which carries the tetracycline repressor gene. This has been developed by Christiane Gatz and colleagues at the Institut für Genbiologische Forschung in Berlin, and is designed as a tightly controlled inducible expression system. Since HMBS activity is essential for haem biosynthesis, if there is only one gene for the enzyme, and if the antisense constructs are effective in switching it off, then they will be lethal. We, therefore, only want to express them once we have sufficient transgenic material.

As far as the Arabidopsis ferrochelatase cDNA is concerned, the sequencing is almost complete. A final year project student, Gillian Millburn, is helping on this now. Transcription and translation of the protein *in vitro* produces a polypeptide of about 40 kDa, consistent with the size of the predicted open-reading frame. We are shortly going to carry out import experiments with this polypeptide. First into isolated pea-chloroplasts, then possibly yeast mitochondria, since although there does appear to be a presequence, it is difficult to determine if it is a chloroplast or mitochondrial transit peptide simply from its sequence. Preliminary experiments show a single message, the same size as the cDNA on northern blots of Arabidopsis leaf RNA, suggesting only one form of the enzyme in this tissue.

> "32 yellow or white seedling-lethal mutants show a normal pattern of synthesis of protochlorophyllide."

Finally, Ashley Cook has screened 32 families of yellow or white seedling lethal mutants of Arabidopsis, and they all show a normal pattern of synthesis of the chlorophyll precursor protochlorophyllide. It is therefore possible to say that none of them are defective in the pathway up to this point, and their lack of chlorophyll is almost certainly epistatic. If you pick up any other plants with the same phenotype, we would be interested to screen those by the same method.

Alison Smith, Michael Witty, Saw »»

Hoon Lim, Ashley Cook & Gillian Millburn; Department of Plant Sciences, University of Cambridge.
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From Mandy Walker...

Trichome development in *Arabidopsis*: cloning of ttg.

We are sorry that we are losing Nigel to the Institute of Biotechnology (just down the road with big plush labs) where he will work with Jim Murray. Paul Davison from Ian Furner's lab has been appointed from 1 February, 1992 and we are pleased to have him filling in for a while.

On the work side of the project, analysis of the recombinants between *ttg* and *ga3* (4 map units proximal to *ttg*) is proceeding with yet more DNA isolations and Southern blots. We are confident that ttg lies quite close to one of the RFLPs in this region. We are checking some more recombinants to confirm this before heading into subcloning, transformation and complementation.

Mandy Walker & John Gray; Botany School, University of Cambridge.
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From Zoe Wilson & Bernie Mulligan...

Genetic male sterility in *Arabidopsis*.

The walking side of the project is now almost up to full strength with the timely appointment of Tania Perehenic (RA), and the keenly awaited arrival of Leonid Shlumukov. We have been spent the last few months consolidating our mapping information around the *ms1* locus. This has been done in a number of ways. First, we have been generating a large number of recombinants in the region between *ms1* and *fg*. These, in combination with the 100 recombinant lines between *ms1* and *ttg* (thanks to Mandy Walker & David Marks) will be used to ascertain the extent of subcloning required from the particular YACs we think span *ms1*. In addition, we have previously used X- and γ-rays to generate a number of *ms* mutants which are allelic to *ms1*. It is likely that some, if not all, of these are deletions. We hope to use these to aid our "homing in" on the exact location of *ms1*. We are currently linking the regions around *ms1* in three YAC libraries (Grill - Columbia and Ler; Ward - Columbia), using end-probes produced by cassette PCR and plasmid rescue. Protocols are being developed for the subcloning and complementation analysis of subcloned YAC fragments which may contain *ms1*.

On the microscopy front, we are really impressed by Regan & Moffatt's paper on the cytological analysis of pollen development in *Arabidopsis* (Plant Cell 2, 877-889 (1990)). Janet Dawson is repeating their methods on our male sterile mutants and we hope to give an update on this work in the next report. Kriton Kalantidis is starting to set up some *in situs* with *Arabidopsis* buds, armed with some anther specific probes, kindly provided by the Goldberg lab.

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GUEST SUMMARIES

From Mark Aarts...

Enhancer trapping with the *En-I* transposable element system.

Winter has brought us some promising results, at least encouraging enough to stay as optimistic as always about the application of *En-I* as an additional system to *Ac-Ds* for transposon tagging.

In the last Newsletter, I informed you that transgenic plants were obtained containing a T-DNA construct on which an *En* element without termini (giving transposase functions) cloned downstream of a 35S CaMV promoter, as well as an *I* element (wildtype, originally isolated from maize), cloned directly downstream of the ATG startcodon of an NPTII gene, rendering it non- functional. The NPTII gene is controlled by a TR 1' promoter. Hygromycin resistance was used as selection marker for transformation. Progeny obtained after selfing of the four independent transformants were used to determine the number of T-DNA loci by scoring segregation of hygromycin resistance loci. Three transformants contained two T-DNA loci and the other one only one. From one transformant with two T-DNA loci, enough seeds were obtained to screen also for kanamycin resistance. Two seedlings out of 20 were full green and five displayed a variegated phenotype. Assuming that in only one in three cases excision of the *I* element will restore the reading frame of the NPTII gene, this gives an excision frequency of 30%. Of course it must be noted that the original transformant had two T-DNA loci with an unknown number of T-DNA copies per locus.

DNA of some hygromycin or kanamycin resistant progeny of the four transformants was used for a PCR test to determine excision on the molecular level. Using primers located on both sides of the cloning site of the *I* element on the T-DNA, excision was expected to give a PCR fragment of about 300 bp. This expectancy became true, since indeed some progeny of two transformants showed this excision band. Two such PCR products (of two different plants of one family) were cloned and one clone of each was used to determine the sequence. After comparison, these sequences turned out to be similar and corresponding to the sequence predicted to arise after excision. The sequence showed that this excision would not restore NPTII activity. Since one of the plants giving this PCR fragment was kanamycin resistant, another excision event must have taken place.

We are currently growing plants to look for reintegration of excised *I* elements. Hopefully, you will read more about this in the next Newsletter.

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From Jim Beynon...

Mapping disease resistance genes to *Peronospora parasitica* and *Albugo candida*.

The cross between Col0 and Nd0 can be used to define two resistance genes: Col0 is resistant to *P. parasitica* isolate CALA, and Nd0 is resistant to *P. parasitica* isolate EMOY2 (see report from Eric Holub). This cross has been used previously in genetic analyses and, hence, the polymorphisms between the parents have been defined. We are using RFLP probes in an interval mapping technique to locate these loci. Eric has produced 66 F_3 families from F_2 plants selected for susceptibility to CALA. Another 59 F_3 families have been produced from F_2 plants selected for susceptibility to EMOY2. Mahmut Tor is currently isolating DNA from these 125 F_3 families to begin the mapping programme to locate both genes.

"Using RFLP probes in an interval mapping technique to locate these loci."

At the same time, we have begun to map a locus for resistance to *A. candida* isolate EMAL using the cross Wein (sensitive) x Kes37 (resistant). Analysis of this cross will be more complex than with Col x Nd0 because neither parent has been analysed previously for RFLPs. Edemar Brose has begun to screen the parental genotypes with currently available probes, using six restriction enzymes and initial results indicate that approximately 50% of the combinations tested detect polymorphisms. Polymorphisms have been detected on all five chromosomes and, therefore, it should be possible to map the locus for resistance to *A. candida* using the method of interval mapping. For this purpose, we have generated over 370 F_3 families from the cross and are screening them with *A. candida* isolate EMAL to identify those that are homozygous for resistance or susceptibility. In this case we will use both homozygous resistant and sensitive plants as they can be easily identified at F_3.

Jim Beynon, Mahmut Tor & Edemar Brose; Dept. of Biochemistry and Biological Sciences, Wye College, University of London.
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From Vicky Buchanan-Wollaston...

Isolation and characterisation of genes involved in leaf senescence.

This programme has made some progress since the last report. Many EMS induced mutants of *Arabidopsis* have been screened and so far two potential classes of mutants in leaf senescence have been identified. In one of these classes, the rate of protein breakdown is affected, with equivalent amounts of protein being found in senescing leaves as in green leaves. In the other class, the plants stay green longer before senescence occurs and in some of these mutants there is a considerably longer flowering period. All these mutants need a lot of further analysis to confirm them as senescence mutants.

The other part of the programme has been to isolate cDNA clones for genes that are specifically induced during leaf senescence. This has been carried out with *Brassica napus*. A cDNA library was made from RNA isolated from leaves at various stages of senescence. This library was differentially screened and clones showing specific expression were identified and rescreened. Several clones have been isolated that are expressed in senescing leaves and not in green leaves and these are undergoing further analysis. Homology of some of these genes to *Arabidopsis* DNA has been using by Southerns.

A genomic library in λEMBL3 for Landsberg *erecta* is available.

We have constructed genomic libraries (approx 10^6 clones each) in λEMBL3 for *Arabidopsis* Landsberg erecta and *B. napus* Falcon. We have not used these libraries ourselves yet, but they are available if anyone wants them.

Vicky Buchanan-Wollaston, Raziuddin, & Ailsa Chambers, Dept of Biological Sciences; Wye College, University of London, Ashford, Kent, TN25 5AH.
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From Michel Caboche...

Arabidopsis in Versailles.

Last fall, we started an Arabidopsis group in Versailles. Started means that we will move into a properly equipped laboratory, downstairs in the plant physiology building, when ready, hopefully next summer. For now, the new group is somewhat scattered in the Institute. Our objectives are to study early steps of plant development, with the emphasis on the control of hypocotyl elongation.

Catherine Bellini will develop a classical approach of EMS mutagenesis and M2 seed screening to identify plantlets with abnormal hypocotyl development (under light or in the dark). Jan Traas will characterise the mutants at the cellular level and try to correlate the effect of mutations with specific (ultra) structural modification in cells and tissues. Emphasis will be put on two important morphogenetic elements: the cytoskeleton and the cell wall. On the molecular side, Thierry Desprez will build a cDNA library of the elongating hypocotyl, and develop a systematic sequencing of the corresponding cDNAs with an Applied Biosystem equipment. David Bouchez would like to map these cDNAs on the different chromosomes, the ideal being to use PCR on an ordered YAC library when it becomes available, but we will need to do this first by RFLP analysis.

The purpose of this approach is in the long run to compare the genetic map of hypocotyl deficiencies to the cDNA map, to identify coincidences, and hopefully find shortcuts to clone the genes of interest. Of course genetic analysis and systematic sequencing have to be exhaustive to reach this point, and the project will take time.... Herman Höfte will join us soon to explore more rapid routes to some of the genes of interest. Last but not least, Hélène Lucas will try to establish a collection of plants carrying insertions of the retrotransposon Tnt-1 from tobacco into Arabidopsis, and this collection when available will be used to tag hypocotyl genes. Hélène will make chimeric constructs of a 35S promoter with the R and U5 domains of the first LTR of Tnt 1, to induce the transcription of the normal Tnt-1 transcript in the germline. She also would like to introduce an insertion marker in the retrotransposon. ➳

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From Ivo Cetl..

Towards exploration and conservation of natural genetic variation in Arabidopsis.

Two systems of natural genetic variation in *Arabidopsis thaliana* have been studied more or less intensively until now: the genetic system controlling the flowering-time differences (Laibach, 1951), and that of allozymes (Abbott and Gomes, 1989). In establishing the Biological Resource Centers, the Multinational Coordinated Genome Research Project also anticipates to collect natural genetic resources or ecotypes. I am especially glad to see that NASC is interested to include them in its programme. I suppose that the activities in this field shall be based on population-genetic knowledge on one hand and to support further development of population genetics on the other.

"...genetic variation not only between populations, but also within populations."

Concerning the flowering-time differences, our experience going back to the early '60s shows that there may be genetic variation not only between populations, but also within populations and, in the latter case, not only between plants, but also within plants, *i.e.*, a part of a natural population may consist of heterozygotes segregating for early and late flowering.

I think that the first task in the exploration of the global genetic variation in flowering time is to classify the population samples according to the three levels of genetic variation. Our material from the central part of Czechoslovakia (Moravia) indicates that only a small part of population samples may be represented by only one homozygous line. The majority of our material is from populations composed of two or more different homozygous lines or even of homozygotes and heterozygotes. In the latter case, several homozygous lines may be derived from the heterozygotes. The best way of conserving the genetic variation in flowering time seems to be to maintain one, two or more homozygous lines according to the level of variation in the original sample. Thus a risky long-termed conservation of original samples connected with inevitable loss of some genotypes may be omitted.

It seems that the genetic system controlling flowering-time differences is adaptive and thus a product of natural selection. The distribution of winter-annual and summer-annual genotypes appears clinal, as dependence on autumnal temperatures and at least a part of genetic variation seems to be connected with balanced polymorhisms especially in the central positions of the cline.

On the contrary, the genetic system controlling allozyme variation appears non-adaptive, heterozygotes are lacking and polymorphisms seem to be neutral or maintained by means of symmetrical selection. While the polymorphisms in the flowering-time system are at least partly balanced and thus "intrazygotic" the polymorphisms in the allozyme system are rather "interzygotic" (Cetl, 1990). The allozyme system allows to follow local and temporal relations recorded in different populations.

The two genetic systems in Arabidopsis natural populations seem promising when we aim to use Arabidopsis as a model object in population- and evolution- genetic research of higher plants, at least of those predominantly self-fertilising.

It would be good to ponder on various ways of exploration and conservation of natural genetic variation and to search for various other systems from visible genes to DNA sequences. I would also appreciate obtaining fresh seed samples collected according to the instructions in AIS 24, 1987.

I hope to initiate effective ideas concerning the activities of colleagues working in the field of natural genetic variation.

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From Michel Delseny..

Still with Em genes....

Extensive progress has been made on the Em genes from Arabidopsis. Two genes have been characterised and shown to be expressed in maturing seeds. A group of 14 cDNAs has now been sequenced and no evidence for more than two genes has been observed. Gene-specific probes have been obtained and used to study gene expression. They reveal a differential time course of expression during seed maturation. Transgenic tobacco and Arabidopsis plants have now been regenerated with a full length promoter of one gene fused to GUS as well as with several deletions. The data confirm that expression of this gene is mostly restricted to the seed embryo.

Weak stimulation of the reporter gene expression was observed in young leaves treated with ABA, but not in the control. Seeds of the transgenics have now been passed to Maarteen Koornneef for transfer to *abi* and *aba* mutant backgrounds. We learnt in Tucson that Ruth Finkelstein (Santa Barbara) has characterised one of these two genes. Fortunately, we focussed our transgenic plant experiments on the other one, but it is fine to be able to exchange information and data: we should progress faster!

Present work on these genes involves construction of new deletions with both types of genes (the sequence of their promoter is fairly different), as well as the hybridisations *in situ*, which are carried out in collaboration with Montserrat Pages in Barcelona.

Besides this work on Em, several other genes are under investigation in Perpignan. Following the characterisation of a new cruciferin gene in radish, we have isolated and characterized several partial cDNA clones of the corresponding sequence in Arabidopsis. Most likely they are representatives of the CRC cruciferin mentioned by Elliot Meyerowitz's group.

Another gene with a cysteine-rich repeat motif has been isolated with a radish cDNA probe. This is a new gene and at least two different cDNAs have been identified in *Arabidopsis*. More information on these genes should come in future issues when

sequencing is completed. Progress has also been made on the T-DNA tagging and promoter-trapping project. More lines have been regenerated by Martine Devic and Patrick Gallois who are beginning to analyse some the most interesting plants.

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From Louise De Villiers..

Senescence in Arabidopsis, the story so far.

Several newsletters ago, I reported on protein and chlorophyll degradation in Arabidopsis induced to senesce in the dark. The results from these experiments were not reproducible due to large variations in the rate of senescence between individual plants. It was observed that the leaves of the Arabidopsis rosette senesced sequentially with age under normal conditions. In other words, at any one point after the initiation of senescence, the stage of senescence of each leaf depends on the position of the corresponding node in the rosette. By determining protein and chlorophyll content in the leaves of individual plants undergoing natural senescence we were able to combat the problem of reproducibility.

I intend to isolate senescence specific DNA from Arabidopsis using differential screening of a cDNA library from senescing leaf tissue. Much of the work I have done so far has involved the quantification of chlorophyll and protein content during the natural senescence of the rosette to determine the stage of senescence at which the cDNA library should be prepared.

Chlorophyll, protein and RNA were extracted from the leaves of the 3rd to the 10th node of the senescing rosette (the leaves of the first node were ascribed to the cotyledons). The levels of chlorophyll, protein and RNA decreased with age of the tissue, and it was shown that *Chla* decreased more rapidly than *Chlb*, but the ratio of *Chla* to *Chlb* remained fairly constant. This suggested that the reaction centre cores of photosystems 1 and 2 and the light harvesting complex were equally stable. It was also clear that there was not a linear correlation between the amounts of chlorophyll and total protein and the age of the tissue. The chlorophyll and total protein levels were considerably higher in leaf nodes 9 & 10 than in 7 & 8. This trend was also shown between leaf nodes 5 & 6 and 3 & 4. However, the changes in levels of chlorophyll and total protein between leaf nodes 7 & 8 and 5 & 6 were minimal. This may indicate that the initiation of senescence occurs before visible yellowing of the leaf.

During senescence, the content of individual proteins associated with the chloroplast was examined using western analysis. The thylakoid associated proteins such as LHCP2, cyt f, D1, D2, psaF and psaD all showed a sudden decline from the leaves of nodes 7 & 8 and 5 & 6 to those of nodes 3 & 4, while the change in protein content between leaves of nodes 9 & 10 and 7 & 8 was less striking. Other proteins studied include the cytosolic and chloroplastic isoforms of glutamine synthase (GSI and GSII respectively), which are involved in nitrogen metabolism. Although both declined during senescence GSII decreased between leaf nodes 7 & 8 and 5 & 6 where-as the decline in GSI was similar to that seen in the thylakoid proteins. GSII was therefore more unstable than GSI.

> senescence may occur before visible yellowing of the leaf

The changes in levels of other proteins such as enzymes of chloroplast biogenesis, papain and RUBISCO showed a similar trend to that seen for the thylakoid proteins.

Cell-free translation products of RNA isolated from senescing leaves were separated using gradient SDS-PAGE and visualised by autoradiography. Polypeptides of 48 and 32 kDa seemed to increase with age of the tissue. However, these results need to be confirmed using the higher resolution of 2-dimensional gel SDS—PAGE.

It is clear from these results that making a cDNA library only from the oldest, most senescing tissue will not necessarily lead to the isolation of sequences specific to different stages of senescence. It is possible that the leaves of nodes 7 & 8 may be expressing genes specific to the onset of senescence, those of 5 & 6 for the process of senescence itself and nodes 3 & 4 for the terminal stages. It will therefore be advantageous to examine the gene expression in the leaves of all the nodes to answer some of these questions.

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From Rudy Dolferus..

Adh in Arabidopsis.

The unique Arabidopsis Adh (alcohol dehydrogenase) gene contains six introns and the coding region shares a high (73%) nucleotide sequence homology with the maize Adh1 gene. The gene displays developmental and tissue specific expression, and is also induced in roots by environmental factors, such as anaerobic stress and the addition of auxins (2,4-D). In addition, we have demonstrated that the Arabidopsis Adh gene can be induced by other environmental stresses, such as cold, wilting, and by the phytohormone ABA.

To identify the regions in the Adh promoter that are important for these various expression patterns (Cis-acting elements), a set of 13, 5'-deletions of the complete Adh promoter (-962 to +58) was made, fused to the GUS reporter gene, subcloned in pBIN19 binary vector, and introduced into Arabidopsis root explants using *Agrobacterium tumefaciens* mediated transformation. In addition, four constructs with mutations, either in regions shown by footprinting *in vivo* to be protein contact points (Ferl & Laughner, 1989), or in the presumptive Arabidopsis anaerobic response element (ARE: 5'-TTGGTTT motif; Olive *et al*, 1990; 1991), were transferred to Arabidopsis root material.

Analysis of the expression pattern of these chaemeric gene constructs in transgenic Arabidopsis plants is currently in progress. This will lead to the identification of those regions in the Arabidopsis Adh promoter important for tissue specificity and control of gene expression under various environmental stimuli, as well as hormonal control of Adh gene expression. In particular, we will determine whether induction of Adh activity by different environmental »

stimuli is regulated through the same or different cis-acting elements. To investigate any steps in the signal transduction pathway leading to Adh gene expression, we have started two additional approaches. In the first place, we started to select for regulatory mutants that fail to express the Adh gene. Transgenic Arabidopsis seeds, transformed with a complete Adh promoter (-962 to +58)/GUS chaemeric gene, are mutagenised using EMS, and Adh- mutants are selected using allyl alcohol as a suicide substrate. These mutants will then be tested for the absence of Adh-promoter-driven GUS activity. In addition, we will investigate whether function and inducibility of the Adh promoter is affected in any of the Arabidopsis hormone mutants: auxin mutants (*axr1-3*, axr *1-12*), ABA mutants (*aba*, *abi 1-3*), and gibberellic acid mutants (*gai*, *ga1-5*). These approaches should allow us to start investigating the pathway of signal transduction leading to Adh gene expression under different environmental stresses.

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From Allan Downie...

Arabidopsis root hairs.

As a group that has spent a lot of time looking at the contortions induced in the root hairs of legumes inoculated with rhizobia, we thought it would be interesting to use some of our time to screen mutants of Arabidopsis for alterations in root hair structure.

"Mutants that appear to lack root hairs."

It has been reported that some mutant lines have root hair deformities similar to those induced on legumes by rhizobia. We have looked at three M_2 populations of mutagenised seed: EMS-treated Columbia and Landsberg (from Lehle seeds) and some γ-irradiated material kindly given to us by Nic Harberd. Among these seedlings we have seen a number of different phenotypes, some of which come through to the next generation. The frequency at which we find clear, inheritable phenotypes is somewhat lower than we expected; especially from the EMS treated seed. Particularly interesting to us are a couple of mutants that appear to lack root hairs completely, whilst others have short and/or deformed root hairs. However, to date nothing we have seen remotely resembles the type of deformations induced in legume root hairs by rhizobia. Nevertheless, we think that some of the mutants we have found may give us the opportunity to understand aspects of the development and unusual structure of root hair cells.

Andrea Davies, Shilpa Ghelani & Allan Downie; John Innes Centre, Norwich.

From Lieve Gheysen...

More worms....

As was already mentioned in earlier Newsletters by Peter Sijmons, not only scientists, but also nematodes love Arabidopsis. In the lab of Genetics in Gent (Belgium), we have started a small research project on the use of Arabidopsis as a model system in plant/-nematode interactions. Our main strategy is to search for mutant plants that are less susceptible to infection by the root-knot nematode, *Meloidogyne incognita*. Andreas Niebel and Janice de Almeida in our group are presently screening several thousand Arabidopsis plants from an E.M.S. mutagenised seed stock. We would prefer to work with diepoxybutane generated mutants, but until now we could not obtain the seeds, or a protocol to make them. Alternatively, T-DNA tagged mutants would also interest us. Can any of you, readers, help us?

Since we like Arabidopsis so much, we intend to use it more and more in our nematode studies. However, current grants have tied the rest of us to working on crop plants for several more years (Walter Van der Eycken on tomato, Mansour Karimi on potato and myself on oilseed rape). Nevertheless, we glimpse at Arabidopsis (our secret love) now and then and try to do some small experiments with it.

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From Barbara Kunkel & Andrew Bent...

Genetic analysis of disease resistance in Arabidopsis: introducing the resistance gene RPT2.

We are using two complementary genetic approaches to identify and characterise Arabidopsis genes that control disease resistance to the bacterial pathogen, *Pseudomonas syringae* pv. tomato (Pst). The first approach is to study the genetic basis of resistance to Pst by crossing naturally occurring susceptible and resistant ecotypes. Ecotype Po-1, which is susceptible to Pst strains expressing the cloned avirulence gene avrRpt2, has been crossed to the resistant ecotype Col-0. The F_2 and F_3 segregation patterns suggest that two separate genes, both of which are required for resistance, are segregating in this cross. Po-1 also appears to lack two genes required for resistance to bacteria carrying the avirulence gene avrB. At least one of the genes required for avrB resistance differs from those effective against pathogens carrying avrRpt2.

Our second approach has been to screen for mutants with altered resistance to Pst. As Roger Innes reported in *For Thale or Went* (July, 1991), one of these mutants has lost resistance to Pst strains carrying the avirulence gene avrRpt2. This mutant, D203, is altered specifically in its ability to recognise bacteria expressing avrRpt2, as it retains resistance to bacteria expressing other avirulence genes. Recently, we have shown that resistance to Pst strains carrying avrRpt2 segregates in a 3:1 ratio in F_2 progeny from a cross of D203 to wild type Col-0. This indicates that susceptibility in the mutant is due to a lesion in a single gene. Identification of a resistance gene in the plant with specificity for a single bacterial avirulence gene demonstrates the gene-for-gene nature of this Arabidopsis/Pseudomonas interaction. We are now referring to the locus defined by the D203 mutation as RPT2.

We are RFLP and RAPD mapping the resistance loci identified by these two approaches. Further genetic analyses are in progress to tie together our work with the suscept-

ible ecotypes and the mutants, giving us a good start on uncovering the plant loci that control disease resistance in the Arabidopsis/Pseudomonas interaction.
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From Mike Jones...

Arabidopsis as a suitable host for plant-parasitic nematodes.

We have been screening the susceptibility of Arabidopsis to infection by different isolates or root-knot nematodes in Western Australia.

"Does anyone have information on *Meloidogyne species?*"

Arabidopsis is virtually resistant to some of these. We are in the process of identifying the species of root-knot nematodes. The low-pathogenic species may be *Meloidogyne javanica*. Does anyone have information on *M. javanica* and other species (*e.g., M. hapla, M. arenaria*) regarding susceptibility of Arabidopsis?
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From Natalya Klueva...

Arabidopsis in the USSR.

Study of the organisation of heat shock proteins in Arabidopsis is moving ahead, though not as rapidly as desirable. Heat shock protein(s), containing the high molecular weight (700 kDa) structure I had reported on previously, has been isolated in quantity great enough to analyse its polypeptide composition by SDS-PAGE. Immunoblotting with anti-HSP 70 antibodies from tomato cell culture (obtained from Prof. K-D. Neumann, FRG) showed the presence of several HSP 70 homologous protein bands. No other polypeptides were detectable as judged by silver staining of the gel. The use of anti-groEl protein antibodies would be preferable to show the absence of reactivity, but they appear not so easy to get.

Alexander Pemov (Plant Physiology Institute, Moscow), is polishing his first data concerning Arabidopsis histone families and will be ready by the next issue.
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From Maarten Koornneef...

Seed dormancy mutants in Arabidopsis.

In the July newsletter, I mentioned our EC seed development and abscisic acid (ABA) project. Karen Leon, who works on this subject, has isolated a number of mutants, whose freshly harvested seeds germinate within 1-3 days on water -- without a cold treatment. Some of these mutants have additional phenotypes such as excessive waterloss or aberrant seed morphology associated with a limited survival upon storage. The latter class of mutants looked very interesting because the respective genes may be crucial in the regulation of seed development. A large proportion of these new non-dormant mutants, including the extreme phenotypes, are alleles of *abi3*. The existence of these extreme alleles is important because it indicates that this gene, which is (being) cloned by Jerome Giraudat in Gif-France is really a crucial regulator of seed development in Arabidopsis.

Through various grapevines, we heard that a Japanese group isolated such extreme *abi3* alleles too. Apparently, Arabidopsis is more competetive nowadays than 12 years ago, when the number of Arabidopsis scientists in the world was about the same as the number of people working on Arabidopsis in an average Plant Science Research Center. Fortunately, we have more mutants to work on. Some of the wilty mutants may be related specifically to ABA biosynthesis or ABA sensitivity. Indeed we found another *aba1* allele, but hopefully new loci will be identified soon.
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From Karin Meierhoff...

Photosynthesis mutants of *Arabidopsis thaliana*.

The development of chloroplasts requires a close interaction of nuclear and plastid genomes. We are interested in the identification of regulatory genes that are involved in the biogenesis of the photosynthetic apparatus. To this end, we are isolating mutants of *A. thaliana* showing high (*hcf*) or low (*lcf*) chlorophyll fluorescence caused by defects in the photosynthetic complexes. So far 70 *hcf* mutants have been isolated in the M_2 progeny of EMS- treated seeds. These are being characterised at the physiological and molecular level.
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From Peter Sijmons...

Worms on weeds!

After infection of Arabidopsis roots with nematodes, specialized feeding structures are induced by the nematode inside the vascular cylinder. The feeding structure can get many times the normal diameter of an Arabidopsis root within several weeks after invasion. Massive amounts of ER and mitochondria accumulate around the stylet (= suction apparatus of the nematode) together with a range of other changes that can be seen in the host cell. In short, cells comprising the feeding structure have now one major mission in life: they must provide food for the developing worm! Although we have no idea how, it is obvious that the gene regulation in these feeding structures is altered to fit the needs of the nematode. We are trying to shed some light in this challenging black box.

Transgenic Arabidopsis lines (transformed with promoterless GUS constructs, both home-made and many lines from Keith Lindsey, Leicester) are now being screened for GUS activity inside the feeding structures. Lindsey and others have already demonstrated the surprising frequency with which regulatory sequences can be tagged through this ➤

approach and indeed we have seen several examples of blue syncytia, although so far the staining is not (yet?) specific for these cells. Still, the results are promising enough to continue the screening. We will keep you informed.

"The feeding structure can get many times the normal diameter of an Arabidopsis root."

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From Brian Thomas...

The lifes of Brians

Photoperiodic Induction

Brian Thomas & Dave Mozley

Since our last report, AFRC student Dave Mozley has been continuing to try and unravel the photoperiodic responses of Arabidopsis Landsberg *erecta*. He has found that as Arabidopsis ages in short days under fluorescent lights it becomes increasingly sensitive to long days. Seven days after germination, four days are required for induction, but by day 17 only one is required. (Similar findings have been made by Rau's group in Munich.) Thus at 17 days, L. *erecta* becomes a single-cycle long day plant. By growing plants under identical short day conditions, except for the single treatment day, it is possible to isolate the effects of light treatments to those on daylength perception and induction, while avoiding any effect of daylength on photosynthesis or general development.

These findings, along with our earlier report that flowering could be delayed by short days given between the second and seventh days after germination in plants grown under otherwise continuous long days, have been used to devise screens for plants with altered daylength perception or induction characteristics. We have several mutants which are not delayed by the early short day treatments. We refer to these as short-day-insensitive or *sin* mutants. The *sin* mutants flower much earlier than the wild type when grown under continuous short days. We also have some that are induced by a single long day when only seven days old (a time at which L. *erecta* requires four long days). We call these single-inductive-day or *sid* mutants. The characterisation of these is less advanced.

The single cycle system is also allowing us to examine the photoperiodic behaviour of existing hy mutants. Both *hy1* and *hy2* mutants show enhanced sensitivity to long days compared with L. *erecta*. It is possible, therefore, that they might show up in the "*sid*" screen. Of the others, *hy3* behaves almost identically to the wild-type, but *hy5* is less sensitive. A single cycle at day 17 is inductive when a short day is extended with far-red light, but not with red light. This is consistent with the photoreceptor being phytochrome in High Irradiance Response mode.

"...short-day-insensitive or *sin* mutants."

On the other hand, supplementing red with blue light gives a strong inductive response. Under these conditions the blue light must be acting through a photoreceptor other than phytochrome. Photoperception therefore, appears to be potentially mediated by two distinct photoreceptors, namely phytochrome and a blue-absorbing photoreceptor. Current work is to characterise further the *sin* and *sid* mutants, both physiologically and genetically. We are also beginning to look at circadian rhythmicity in the inductive response to light.

UV-B and plant development.

Brian Jordan, Richard Anthony & Pat James.

Supplements of ultraviolet-B radiation to growth cabinet lighting cause a rapid change in the pattern of Arabidopsis gene expression. The mRNA transcripts for rbc S and cab decrease to low levels after 24h exposure to UV-B radiation. In contrast to the down-regulation of gene expression for chloroplast proteins, mRNA levels for chalcone synthase increase dramatically within four hours. The increase in chalcone synthase corresponds to an increase in flavonoid pigment synthesis. The chlorophyll composition, however, does not change significantly even after days of UV-B treatment, which contrasts to more sensitive species such as pea.

We are now using RACE-PCR to isolate other genes involved in the UV-B defence response and also mutants to investigate the signal transduction mechanism.

The Two Brians; Horticulture Research International, Littlehampton.

From Annette Vergunst...

About auxins and gene targeting.

A continuation of the auxin signal transduction and gene targeting projects....

The aim of Eric's project is to obtain and analyse transgenics with a mutant phenotype that is correlated with overexpression or disruption of phyto-hormone and morhogenesis-related genes. He is still optimising the selection criteria for regeneration of transgenic tobacco harbouring a promoter-out construct.

The analysis of transgenic plants containing the auxin inducible (tobacco) promoter of pGNT hooked up to GUS, is being extended by Dianne vd Kop. Expression of the GUS gene becomes visible after histochemical staining in the lateral root cap. Expression increases after germination of seeds on media containing 2,4-D, NAA or IAA. Four days after germination GUS expression disappears. It reappears when lateral roots are formed. Expression was only visible in progeny of a heterozygous parent. The progeny of a homozygous parent gave no blue staining. A homozygous plant was backcrossed to wildtype to see whether the progeny gives expression of GUS. Furthermore, crosses with hormone mutants have been made to see if there is any change in the expression pattern.

In the gene targeting project, Stephan Ohl is facing some difficulties getting alpha-amanitin selection to work. The aim is to target the RNA polymerase II (AtrpII) gene of Arabidopsis. Wildtype plants should have an amanitin-sensitive phenotype. A single aminoacid change confers amanitin resistance. The drug is able to kill animal cells in very low amounts (1 µg/ml), but plant cells appear to be quite resistant. The problem seems to be related to bad uptake of alpha-amanitin. Experiments are being conducted to increase the level of uptake.

As mentioned in the Nov. issue, the major problem Annette Vergunst was facing in setting up Arabidopsis protoplast co-cultivation with *Agrobacterium*, was the necessity of alginate embedding directly after isolation for good regeneration. There now seems to be more positive outlook as to how to tackle this problem. Two directions are being followed: (i) use of different hormone regimes to enable culture of protoplasts in liquid medium during the first days of culture and, (ii) use of 2-6 dichlorobenzonitril (2-6DB). This is a cellulose biosynthesis inhibitor (which doesn't inhibit nuclear division) and should make it possible to "extend" the time during which protoplasts can be kept in liquid culture without losing competence for regeneration. The problem so far is partial cell-wall formation which gives rise to "budding." At this time there are no clear results and so before going into detail many more experiments will have to follow....
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From Manuel Talón...

Studies of hormonal regulation in *Arabidopsis thaliana.*

Using *Arabidopsis* as a model system, we are studying the mode of action of plant hormones in the regulation of plant growth and development. The aim of our programme is to combine physiological and molecular approaches to: (i) isolate mutations affecting important hormonal processes, (ii) characterise these mutations, and (iii) identify genes involved in hormonal biosynthesis, and in the signal transduction pathway.

We have been engaged in the physiological characterisation of the gibberellin (GA) deficient mutants: *ga1, ga2, ga3* (Zeevaart & Talon, 1991), *ga4* and *ga5* (Talon, *et al.*, 1990a), and the GA-insensitive mutant, *gai* (Talon, *et al.*, 1990b). The immediate objective was to determine the steps of the GA biosynthetic pathway blocked in these mutants. Further, we plan to perform molecular genetic studies to characterise the genes affected by these mutations. Strategies to clone these genes are being developed. We are also interested in studying the relationship between GAs and stem elongation in mutants with altered stem growth, including *cp2, cp3, dw1, le (pa)* and the photomorphogenetic mutants *hy1, hy2, hy3, hy4, hy5* and *hy6*. On the other hand, we are also developing strategies to isolate by direct screening slender mutants, since this type of mutant has not been described in *Arabidopsis* yet.

A second area of interest deals with the rôle of ethylene in plant growth, development and senescence. We have isolated several ethylene-resistant (*er*) mutants showing insensitivity to exogenous ethylene or to its metabolic precursor ACC (1-aminocyclopropane-1-carboxylic acid). These mutants display a wide range of altered physiological and biochemical ethylene responses (hypocotyl and root elongation, germination, leaf yellowing, petal abscission, and ethylene biosynthesis, among others). The mutants are promising experimental systems to examine the rôle of ethylene in different developmental processes. Using a homozygous mutant we have studied the rôle of ethylene in leaf senescence and the interaction of ethylene, abscisic acid and citokinins in the regulation of this process (Zacarias & Reid, 1990). We have also used the mutant to evaluate the involvement of ethylene in root elongation and penetration through mechanical impeded surfaces. These mutants offer the possibility to study without interferences the rôle of ethylene, and their interaction with other plant hormones in the regulation of many physiological processes and stress responses.

The isolation of specific genes involved in these processes is now feasible in *Arabidopsis thaliana*.

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The next newsletter deadline is:
Monday, 5th October.

From A.R. Roubelakis-Angelakis...

Plant Morphogenesis *in vitro:* Molecular Approaches

SEPTEMBER 6-18, 1992, Advanced Study Institute, sponsored by NATO and FESPP, under the auspices of the University of Crete, and the Institute of Molecular Biology & Biotechnology Crete, Greece

The Institute is designed to present state-of-the art plant morphogenesis with emphasis *in vitro*. The topics include Molecular Mechanisms, and Physiological and Biochemical Aspects of Plant Morphogenesis *in vitro*. Lecturers include: S. de Vries (Netherlands), A. Galston (U.S.A.), P. Hatzopoulos (Greece), D. Ry Meeks-Wagner (U.S.A), Y. Meyer (France), M. van Montagu (Belgium), A. Theologis (U.S.A), T. Thorpe (Canada) A. Tiburcio (Spain), K. Tran Thanh Van (France), D. Osborne (United Kindom), I. Vasil (U.S.A), R. Wyndaele (Denmark), and K.A. Roubelakis-Angelakis (Greece).

The Seminar is limited to 100 participants. A poster session will be held and limited oral presentations will be selected. Funds are available to subsidise partially transportation and/or living costs mainly for post-doctorals and graduate students under 28. For further information and application forms please contact the director of the ASI-Seminar: Prof. K.A. Roubelakis-Angelakis; Dept. of Biology, University of Crete, P.O. Box 1470, 71110 Heraklio, Crete, Greece. Tel: 81-232156 ext. 286; 81-236853 ext. 286; FAX: 81-233669.

From Sue Albini...

Dutch Workshop & Symposium on Meiosis

THIS IS A report on the Workshop & Symposium on Meiosis, held by the Dutch Genetical Society at the Agricultural University at Wageningen, The Netherlands, 27-29 November.

On 29 November last year, the Dutch Genetical Society held a one day symposium on meiosis. I was invited to attend a two day workshop on "The Initiation of Pairing," which took place just before the symposium. Thanks to a subsidy from the PMB travel fund I was able to attend.

The workshop was hosted by Christa Heyting, and about fifteen people from around the world participated. The purpose was to sit down and thrash out views and ideas about how homologues pair during prophase I of meiosis. Due to, as yet, a lack of hard experimental evidence this area is wide for debate and can be controversial, this meeting was certainly very lively. The range of pet organisms varied and included yeast and other fungi, *Drosophila*, mice and rats and higher plants. Participants each a short presentation which was intended to raise questions and initiate discussion. A brief overview of one or two of the issues raised follows:

The current research in yeast is extremely sophisticated and it is now possible to correlate biochemical, genetical and physical events throughout prophase I of meiosis. By setting up timecourse experiments for normal yeast and for a range of mutants, Nancy Kleckner and co-workers have shown that double strand breaks in the DNA (precursors of recombination events) appear before the synaptonemal complex (SC) and mature recombinants appear after the SC has been dismantled. The central debate raised by this work is whether or not the SC has a driving rôle during prophase I and brings about homologous chromosome pairing and recombination or is a product of these processes and has a different function.

The work done in Birmingham on Arabidopsis provoked some interesting discussion. Comparing the mean synaptonemal complex length of Arabidopsis and other plants with a small genome size with that of plants with large genomes shows that species with low DNA amounts have, relatively speaking, an enormous, long SC. Compare Arabidopsis with a pgDNA/µmSC ratio of 1:500 to *Allium cepa* where the same ratio is 1:42. This questions any idea that the SC is merely a supporter of the DNA and fosters the idea that the SC has a controlling or specific role. This may be further investigated by examining the interaction of the DNA with the SC during prophase I. One way of doing this is to perform DNA-DNA *in situ* hybridisation to SCs at all stages of prophase I, to determine how specific DNA sequences interact with the SC. This technique has recently been developed for rats and mice, by Peter Moens in Canada, and can now be done in plants (see my project report).

The answers to the questions about the rôle of the SC in homologous chromosome pairing will be found by a merging of cytogenetics, molecular biology and biochemistry, with the synaptonemal complex as the interface between the disciplines. ALBINISM@UK.AC.BIRMINGHAM.COMPUTER-CENTRE.IBM3090

From Jim Beynon...

ARAPNET in Köln

THE FIRST ARAPNET (*Arabidopsis* Pathology Network) meeting was arranged by Jeff Dangl and held in Cologne from November 20-23, 1991. It was originally intended to be a small gathering, between the groups of Alan Slusarenko, Ian Crute, Mike Daniels and Jeff, to develop programmes designed for EC funding. However, the concept of a "Pathology Meeting" spread and Jeff had an escaping genie on his hands. With the often repeated words "absolutely no more people" the meeting grew to around 50 participants.

Nematodes set the scene for the very high quality of all the presentations at the meeting. The potential for using *Arabidopsis* for studying nematodes was discussed. A major advantage is that the infection process can be visualised *in situ*, in a dynamic sense, as there is no need to section the roots. This allowed Florian Grundler to walk away with the award for the "Most Visually Exciting Presentation" with his video of nematodes infecting *Arabidopsis* roots.

Bacteria took the stage for the remainder of the first day. Jeff Dangl reported on progress towards cloning a gene (RPM1) in Col-0 for resistance to *Pseudomonas maculicula* strain M2 and showed that his group were within 200kb of the locus. Brian Staskawicz showed that *avr*Rpt2 was recognised by resistance genes in *Arbaidopsis* and that two genes were required for resistance in a Col-0 x Po 1 cross and these were being mapped. Fred Ausubel discussed a wide range of projects ongoing in his laboratory that include searching for plant mutants that no longer respond to *avr* genes, defence related genes and accelerated death mutants. Mike Daniels and Jane Parker described the establishment of gene-for-gene relationships with *Arabidopsis* and pathovars of *Xanthomonas campestris*. Ulla Bonas reported on the structure and function of *avr*Bs3 where the number of repeated sequences within the protein determine its *avr* phenotype. Finally, Shauna Somerville described an indole thiazole phytoalexin that turned out to be structurally similar to Camalexin.

On the second day Thomas Debener described his work using RFLP probes to analyse how related are *Arabidopsis* accessions. Imre Somssich reported on the use of elicitor induced genes from parsley to obtain similar clones from *Arabidopsis*. Eric Ward described steps towards the genetic dissection of induced resistance and Jonathan Jones detailed the availability of Recombinant Inbred lines for the Col-0 x La-er cross and the development of a RAPD map that will facilitate gene mapping and isolation.

Finally the fungi took the stage. Ian Crute detailed the wide range of resistance genes that have been determined in other plant systems and the implications for *Arabidopsis*. Alan Slusarenko described the wide range of fungi that can infect *Arbidopsis*. They are mapping a resistance gene to

Peronospora parasitica and he described the use of dichloroisonicotinic acid to induce resistance to *P. parasitica*. Eric Holub described results from a half diallele cross between eight *Arabidopsis* accessions and two isolates of the pathogenic fungus, *P. parasitica*. Several potential avirulence and resistance gene interactions were detailed and two resistance genes were actively being mapped in a Col-0 x Nd-0 cross.

The programme also included social interludes that seemed to revolve around barrels of Kolsch that were always quickly emptied. Jane Parker rescued us one night with a tour of her numerous old drinking haunts, even if the most dubious had been closed down since her last visit. On the final night our "disappointment" at the lack of a belly-dancer at the Turkish restaurant was somewhat compensated for by John Mansfield on the spoons. All in all the meeting allowed for the free flow of information and the establishment of a coherent structure to work on the pathology of *Arabidopsis*. Jeff Dangl and all his group must be given credit for the great success of this meeting.
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From Mary Anderson...

Arabidopsis Databases

TWO AMERICAN Databases, which are independently funded, have been established for the development and administration of international *Arabidopsis* resources. Both databases will share the same information, but the emphasis placed on different pieces of information will be different. They will prove to be invaluable resources for the *Arabidopsis* research community.

AATDB ("an *Arabidopsis thaliana* database") is supported by a grant from the U.S. Department of Agriculture through the National Agricultural Library and is part of a large genome database/informatics programme to produce databases for wheat, soyabean, maize, pine and *Arabidopsis*. AATDB is derived from "ACEDB" ("a *C. elegans* database"), which contains *C. elegans* genome database information, and was designed by Richard Durbin (MRC, UK) and Jean Thierry-Mieg (CNRS-CRBM, France). AATDB is being developed at Massachusetts General Hospital, Boston by Howard Goodman, Mike Cherry, Sam Cartinhour and Brian Hauge. AATDB is presently a stand-alone system, but will also feed into a central database at NAL, which will function as the central access point for all the plant genome information. Access will be through Internet, electronic mail and direct dial up. AATDB will be available free of charge, on disc, or via anonymous ftp from several computers, to be used on SUN Microsystems SPARC, or similar UNIX, workstations with 50 megabytes of disc storage space. In time, a Macintosh version of the database should also be available, but this awaits the Macintosh version of ACEDB. To use this version you will require a colour monitor, large hard disc or CD-ROM and a Mac II or better.

AATDB has been designed to be very user-friendly. It is possible to retrieve information intuitively by clicking on objects and icons with a mouse instead of by typing query-language commands, although there is a query language for those who prefer it. Extensive links between different classes of information make it possible to locate interesting data from many different starting points, which is very important when searching informally. Information can be retrieved in simple text or PostScript form.

AATDB already carries the physical map (15,000 cosmids and YACs); the genetic map (morphological and RFLP markers); our (Nottingham) seed catalogue; 1800 bibliographic references cross-referenced by author, journal and keywords; over 200 sequences from genbank; our (Nottingham) subscribers List and the *Arabidopsis* Newsgroup Subscribers List; the "green book" (Meyerowitz and Pruitt); scanned images of RFLP phenotypes and mutant plants. Eventually it will include, amongst other things: 2-point cross data; 3-point cross data; deficiencies, duplications, translocations. Early "prototype" versions of this database will be available shortly.

The second database system, called AIMS (*Arabidopsis* Information Management System), is being developed as part of the Ohio *Arabidopsis* Resource Centre, by Sakti Praminik at Michigan State University and is supported by the NSF. It will be developed using the Sybase system and will be on-line, so no special workstation will be required. This database is orientated towards the Stock Centre needs and has been created with their needs as the priority for design.

Fortunately for us, this database like AATDB, has also been created with the end-user in mind. It will be possible for several individuals to access the system simultaneously. Once logged on it will be possible to use query language or a multiple window method, in the same vein as AATDB, for navigating through the different layers of information. This is very exciting for us. It will revolutionise the way that the Nottingham Stock Centre is administrated. As part of the collaboration with the Ohio Stock Centre we shall be feeding all our information into this database. It will allow you, the user, to get up-to-date information about the stocks that we carry. Not only will you be able to tell what lines we have, but whether a certain line is available and immediately place orders. It is hoped that an early version of AIMS will be on-line sometime in April. The full system should be operational by the autumn.

The organisers of both database systems have canvassed the *Arabidopsis* community for information. However, if you have any strong opinions send them to Boston or Michigan now. There are no firm plans to produce a separate European database. It is up to us to help where possible. One way to stay in touch with the development of the databases is to subscribe to AATDB. This also gives you the opportunity to take place in a discussion forum as individual points, such as nomenclature, are debated. Sakti Pramanik also produced a questionnaire to establish the users needs for a stock centre orientated database. The ➳

results of this questionnaire were posted on the *Arabidopsis* Bulletin Board on January 29. I have a copy of this if anyone is interested and does not have access to electronic mail.

As a final note, if you have any information that you feel should be in the database, however trivial, I strongly encourage you send it in to AATDB or AIMS. The more published and unpublished data that is available in the database, the more powerful the database becomes. Like the Stock Centre, the success of these ventures is dependent on the input from the *Arabidopsis* scientific community, so that means -YOU. Contact addresses:

AATDB: Mike Cherry
CHERRY@FRODO.MGH.HARVARD.EDU
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CARTINHOUR@FRODO.MGH.HARVARD.EDU
Dept. of Molecular Biology, Massachusetts General Hospital, 14 Fruit Street, Boston, MA 02114, USA.

AIMS: Sakti Pramanik PRAMANIK@CPS.MSU.EDU
or AIMS@GENESYS.MSU.EDU
Department of Computer Science, A729 Wells Hall, East Lansing, Michigan 48824-1027, USA.

Randy Scholl ARABIDOPSIS+@OSU.EDU
Arabidopsis Biological Resource Center at Ohio State, 1735 Neil Avenue, Columbus, Ohio 43210.

From Penny Maplestone...

AFRC Plant Molecular Biology Programme: Phase II

Further Particulars for Applicants

Background

1. MOLECULAR BIOLOGY underpins many important current plant science research programmes. Major breakthroughs have been made in recent years and many more are foreseeable in the next decade. The discipline is poised at a stage where the techniques being developed will soon allow the routine identification and isolation of genes of agricultural importance. Technologies developed with model systems such as *Arabidopsis* will be transferred to economically important species for biotechnological development of new crop varieties with specific desirable attributes such as pest and disease resistance; modified physiological responses to environmental variables including daylength and temperature; altered metabolic pathways to produce novel storage products for new food and non-food uses; and new processing characteristics. In addition, there are tremendous scientific opportunities to advance fundamental knowledge of the processes of gene regulation in the control of development and metabolism of the whole plant. This is important not only to increase understanding of the biology of plants, but also because it is only with this understanding that there can be full exploitation of plant molecular biology for the genetic modification of crops.

2. The AFRC's Plant Molecular Biology programme has helped to establish the UK as an internationally competitive nation in this field. The injection of substantial funds over three years has enabled a body of expertise and experience to be built up. It has helped to train a new generation of post-doctoral scientists, has prompted the return of some young scientists from overseas and attracted several scientists from other disciplines to work with plants for the first time. The *Arabidopsis* programme is internationally recognised as a rôle model for other countries developing similar national programmes and UK scientists are amongst the world leaders for work on gene identification, mapping and isolation technologies. Significant advances have also been made in studies of plant metabolism and reproductive processes in species other than *Arabidopsis*. It has proved to be a cost effective use of resources and raised the awareness of the value of collaboration and an interdisciplinary approach.

3. Through the programme's infrastructure of coordination, scientists have been encouraged to attend annual discussion meetings, exchange ideas, materials and information and have had access to a central travel fund for visits to other laboratories at home and abroad. Those involved in the *Arabidopsis* programme have benefited from central resources including a seed centre, quarterly Newsletter and protocol book. These arrangements have helped to build a sense of community, encouraged collaboration, avoided duplication of effort and undoubtedly facilitated rapid progress within the programme as a whole. At international level, the programme coordinators have kept the UK community in close touch with developments in other countries.

Remit of the New Programme

4. Considerable progress has already been made towards the objectives of the first Plant Molecular Biology programme, but much remains to be done and the field of scientific opportunity widens with each new development. AFRC therefore announces a new coordinated programme which will continue the successful technology development with increasing emphasis on the transfer of that technology to crop plants. New research will exploit that technology in the agriculturally important and scientifically competitive areas of strength emerging from the current initiative. These areas have been identified as follows:

(i) Plant reproduction;
(ii) Vegetative development;
(iii) Disease resistance/plant microbe interactions;
(iv) Genome analysis;
(v) Regulation of metabolism/metabolic control;
(vi) Responses to environmental cues.

5. Some of these areas will be best studied in *Arabidopsis* and some more appropriately in other species including some of agricultural significance. The programme will be funded for 4 years at a level approximately half that of the current programme. The exact size will be determined by the number of first-class research proposals received as competitive bids from the scientific community in institutes and HEIs.

6. *Applicants for research grants in category (v),* whose application falls in the area of **"The Biochemistry of Metabolic Regulation in Plants"** should note that their proposals will be considered as part of a joint programme with SERC, continuing the programme of that title initiated last year. The aim of this programme is to promote studies of *biochemical* aspects of the regulation of plant metabolism and the rôle of compartmentation in its organisation and integration. The emphasis will be on studies at the biochemical rather than genetic level of regulation and will therefore include applications in the

following categories:

- Studies of central metabolic pathways in plants
- Studies of other pathways fundamental to plants and their interactions with central pathways
- Studies of the rôle of compartmentation in the organisation and integration of metabolism in plants.

Applicants for this part of the programme should submit their research grant proposals on SERC or AFRC application forms, clearly marked "**Metabolism**" (see para 11 for further details).

Studentships

7. Most of the funding for PMB II will be for research grants, but training will also be an important part of the programme. Funding for research studentships will be available. Prospective supervisors seeking to apply for studentships should do so on the appropriate AFRC studentship application form (marked "**PMB**"). Studentship applications may be linked with particular research grant applications, but a studentship *should not form part of a research grant application.* Studentships will run for 3 years to be completed by September 1997.

Fellowships

8. Support for a small number of post-doctoral research fellowships will be considered. Applications should be made on the standard AFRC Fellowship application form (marked "**PMB**"). AFRC's normal terms and conditions for fellowships will apply under the PMB programme. Fellowship awards must be taken up by January 1993 or as soon as possible thereafter.

Coordination

9. Coordination has proved very effective on the current programme and some funds will be retained centrally for this purpose. All successful applicants will be expected to agree to participate in coordination activities, for example to attend annual meetings and to submit progress reports as and when requested. Applicants may request some travel funds to support research collaborations, but funds for overseas visits and conferences will be retained and administered centrally, as in the current programme.

Procedure

10. Applications are invited from scientists in UK Higher Education Institutions, AFRC Institutes and Horticulture Research International. AFRC's standard criteria of eligibility apply. As this is a 4 year programme, it is anticipated that most research grant applications will be for 4 years, starting on or after 1 October 1992. However, proposals for 2 or 3 years funding will be considered where this timescale is appropriate to the science. Research studentships will be held in the usual way, for 3 years and postdoctoral fellowships for 5 years. Application forms for research grants, studentships and fellowships are available from, Mrs. Katherine Chapman at AFRC Central Office (Tel: 0793 413228). Please contact Mrs. Chapman for any help with completing the new style application form. *The closing date for research grants and studentships is 1 April 1992 and for postdoctoral fellowships is 31 May 1992.*

Special note for Applicants for the Biochemistry of Metabolic Regulation Programme (para 6 refers)

11 As the Councils' applications procedures have now been harmonized, you may apply to *either* SERC or AFRC for this joint programme, though applicants from AFRC Institutes are asked to apply to AFRC. SERC application forms are available from, and should be returned to, Miss Lucy Watson at SERC, Polaris House, North Star Avenue, Swindon SN2 1ET (Tel: 0793 411421). Similarly, for AFRC applications, the contact point is Mrs Katherine Chapman at AFRC (see above). Applications for *research grants* are invited for the joint programme. AFRC will also consider applications for studentships and fellowships in this area. Applications for this programme will be considered by a joint AFRC/SERC peer review panel. *The closing date for applications is 1 April 1992* (except AFRC fellowships, when it is 31 May 1992).

Special note for Current PMB Grant Holders

12. Applications are not restricted to existing grant holders, but some special considerations apply where applicants have current awards.

(i) There will be only one call for applications under the new programme. If you wish to seek a continuation of funding for an existing PMB award, you should submit this by 1 April 1992 despite the termination date of your current award.

(ii) Your application for continued support should be for a maximum of 4 years and, where possible, should end not later than 30 September 1997. You may wish to write a full four-year programme, including a revised plan for the period of your current grant remaining or you may prefer to submit a proposal for a continuation starting from the date of conclusion of the existing grant. In either case, the funding for your current grant is secured until its termination date, regardless of whether or not your application for continuation is successful.

(iii) If you are an existing grant holder applying for a *new* project, your proposal may be for a maximum of 4 years starting on or after 1 October 1992.

(iv) If you hold a current PMB or other related AFRC research grant, you should submit a brief progress report on that grant with your application in the usual way.

Note for Institute Applicants

13. Your application should be submitted through, and countersigned by, your Institute Director. The application should include provision for the costs shown in the application form and *not* the full economic costs.

Helpline

14. If you wish to discuss a proposal ahead of submission, you should contact Miss Susan Riley in AFRC's Higher Education Branch (Tel: 0793 413366).

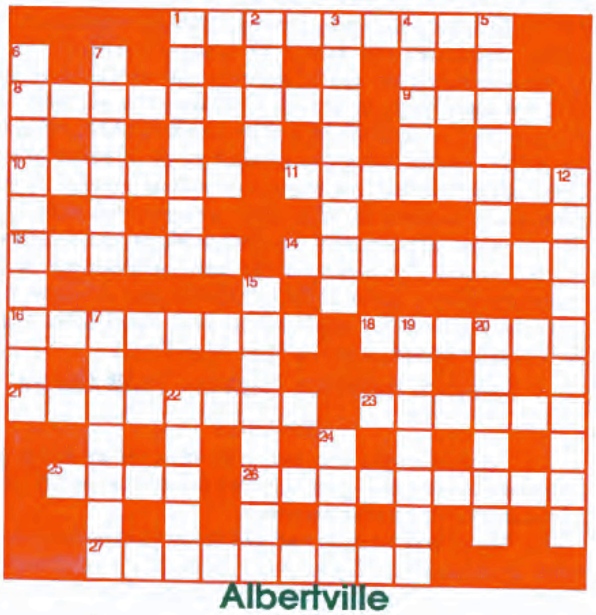
Thanks to...

Sue Albini, Barrie Allen, Mary Anderson, Jim Beynon, Jon Clarke, Jeff Dangl, Laura Donohue, Terry Donohue & Richard Mitchell (The Underground Grammarian), Elucius, Joan Green & Wendy Foster, Penny Maplestone, Black Rot, and Renate Schmidt.

This Issue's Quote

"The disk's in the mail. I'll FAX you a copy this afternoon." *Anon.*

Arabidopsis Prize Crossword



Albertville

by Black Rot

THE DRAW THIS TIME was performed by Clare Lister, who was interrupted from doing 50 mini-preps by the ACM sticking a large box, containing several pieces of paper, under her nose. Those drawn out of said box, were **Penny Maplestone** from HQ, who wins the Newsletter £5 book-token; and **Jo Ross** of the Cambridge Lab. at the John Innes Centre, who wins the site prize of a bottle of Lithuanian Chablis. Both are regular entrants, which goes to show that persistence will be rewarded (eventually).

The unlucky runners up included: former winners, Barrie Allen (Photography, J.I.C.), Brian Forde (Rothamsted), and Alison Smith (Cambridge Univ.); regular entrant, Mary Anderson (Nottingham) [keep trying], and newcomers, Keith Chater, and Lesley Fish & Mark Atkinson (all J.I.C.) -- congratulations to all of them.

In order to try to keep the regulars happy and to try to encourage yet more fresh blood, Black Rot has prepared another entertaining, but still relatively simple crossword. In this, the clues, 1, 8, 9, 11, 18, 21, 23, & 27 Across and 6, & 12 Down are all related to each other by a theme suggested by the title to the title. The usual prestigious £5 book-token for the first correct entry out of the draw.

Clues Across

1. See 23
8. Crust near completion for rapid (downhill) route (6,3)
9. Single seater for 8 falls back into huge gully (4)
10. As confused as Moll about tortuous descent (6)
11. Ski - shoot ? (8)
13. Melted 7 out east in hot land (6)
14. What ER does for F in D-G- REG- F-D- ? (6,2)
16. Test taker finds organic compound in Devon river (8)
18,26. Elevated Greeks, postseason competitors at Albertville (6,9)
21. Get the bends if kilt is disturbed on cableways (3-5)
23,1. Slippery customer gives the northern lass a shilling to dispatch, we hear (3-3,9)
25. Bizarre artist (4)
26. Remove the nerves (9)
27. See 18

Clues Down

1. Marry, wed (7)
2. Inter (4)
3. Plant cell type puts church in pulse field (8)
4. Small island (5)
5. Bargained (7)
6. Those close to the edge on thin support ? (3,7)
7. Italian ice-creams (6)
12. Scratched ? - a hundred up on part of 1 or 9 (3,1,6)
15. Make payment, repay debt (6,2)
17. Agitatedly in music (7)
19. Towards the centre (7)
20. F14 or male Persian (3-3)
22. Glacially, coldly (5)
24. Palindromic pal of the king of Siam (4)

Here are the answers to *At Number Ten*, the crossword in *A-rap-a-bop-sis*:

Stanzas for Scientists

OUR CATERING correspondent, Elucius, has lately been spending much time in his library. Hence, he has chosen a poem, rather than a recipe. For all you ageing fans of Radio One's John Peel, we present a poem by Ivor Cutler from Private Habits (Arc publications 1981).

Ladies Plaits

It has been asserted recently that ladies put their
hair into buns to try to get men
to realise that they too were sapient,
the bun being an overflow skull
for a superabundant brain.
Men invariably accepted the bun as silly ornament,
unable to follow the reasoning,
and the bun fell into desuetude.
Now, some scientists are trying to correlate
ladies' plaits with DNA,
but they have their work cut out.