# ARABA-DABA-DOPSIS!

## The Fourth AFRC PMB *Arabidopsis* Newsletter

## November 1990

In This Issue:



Plus – attached:



## Stop Press

Good news hot off the press for all you chromosome walkers. In a recent e-mail message to Caroline, Chris Somerville reports; "Inhwan Hwang, in Howard Goodman's lab., has done a big walk on chromosome three and thinks that only 10% of the YACs in Erwin Grill's library are chimaeric."

Also, Keith Roberts wants to know if anyone has a reference for the anatomy of *Arabidopsis* roots.

## Thanks

A big thank you to those of you who got your project reports in on time, i.e. over 70% of you. This certainly made production of *Araba-Daba-Dopsis!* a little less difficult, particularly as the vast majority came *via* e-mail or on disc. Most of the rest were only a couple of days late and only one person needed threatening 'phone calls. Please keep up the good work for next time. In the meantime, the ACM wishes all newsletter readers a Merry Christmas and Araby New Year. Although he hopes to see many of you at the Annual AFRC PMB *Arabidopsis* Programme Meeting in Nottingham from December 17-19th.

Wherever possible, please send all contributions to this newsletter, by e-mail (non-U.K. e-mailers may need to reverse the order of the components of the sitename), or failing that, 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 word processor format *and* as a text-only (ASCII) file. Whatever the disk, please 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*.


***Araba-Daba-Dopsis!***: the fourth AFRC PMB *Arabidopsis* Newsletter, November 1990.


Assistant Circulation Manager (ACM), David Flanders
The Cambridge Laboratory, The John Innes Centre for Plant Science Research, Colney Lane, Norwich, NR4 7UJ, U.K.
Tel: 0603-52571 (switchboard) or 0603-502200 (direct line). International +44-603-52571/502200.
FAX: 0603-56844 or 0603-502270. International +44-603-56844/502270. E-mail: ARABIDOPSIS@UK.AC.AFRC.JII

## *Arabidopsis* Awareness

A main feature of the attachments to *Araba-Daba-Dopsis!* is the *Arabidopsis Current Awareness List*. This has been kindly compiled by Joan Green, Head of Library Services at the John Innes Centre for Plant Science Research. The list is derived from Current Contents and is similar to her other "Current Awareness..." lists that you may be familiar with. The first edition covers the period from mid-June until mid-October. Updates will be provided to subscribers with each issue of the *Arabidopsis* Newsletter.

To subscribe to the *Arabidopsis Current Awareness List* costs a mere £10 a year, although it is *free* for anyone in receipt of an AFRC PMB *Arabidopsis* Grant. In order to continue to receive the list with subsequent *Arabidopsis* newsletters, please fill in the form on the back of the list (please return a form even if you are entitled to it free, as Joan needs to keep track of where it's going to) and send it to the ACM at the usual address. Please make cheques payable to "The John Innes Institute."

To find out why the ACM is holding a Magenta pot for Dick Flavell (right), turn to The BackCross Page (15).

## Dave & Dee Learn How

*From Dee Rawsthorne...*

*(Dee is personal assistant to Dick Flavell and editor of the monthly Institute of Plant Science Research News Bulletin. In addition to letting the ACM pinch her article and headline in order to save him writing one, she was also kind enough not to mention his navigational skills, which resulted in us entering Cambridge east from the M11, after travelling west from Norwich.)*

Together with David Flanders, ACM of the *Arabidopsis* Newsletter, I recently went on a course in Cambridge on How to Design, Write and Edit Newsletters. I'm afraid we were both outclassed by the likes of ICI, The Royal Mail, The Sedgwick Insurance Group, and Cornhill Publications to name but a few, who obviously had limitless budgets, staff, DTP's and time to devote to their respective newsletters. A sharp contrast to both Dave and I who try frantically to get each issue of our newsletters out on virtually non-existent budgets, and depend heavily on the availability of a working photocopier!

However, despite the "worlds apart syndrome," Dave still suffering from his recent knee operation, me going through two boxes of tissues due to the onset of a heavy cold, and having to eat a Large Deep Pan Supreme Pizza in five minutes due to the inefficiency of the Cambridge branch of a certain well known Pizza fast food Hut, we both learnt a great deal (including how to overcome acute indigestion).

Whether we will be able to apply successfully all we learned remains to be seen....

## Loyal Opposition

The ACM hopes that regular readers of this newsletter, will be pleased to hear that this edition is, for the first time, being distributed to the "General" half of the AFRC's PMB Programme. Incidentally, their Annual Meeting is scheduled to take place in Reading on 24 March .

Articles are more than welcome from any of our new readers.

## Papers, Protocols & Projects

Got a paper coming out on *Arabidopsis*? Got a good or unusual or even mundane protocol suitable for *Arabidopsis* work? Well, send them in to the next newsletter, which is due out in mid-February. Who knows who will read it? Please submit project reports and any other items by **Monday, 4th February**, at the very latest. The earlier the better, particularly for protocols, *etc.*

## Thanks too...

- Barrie Allen for offset-litho printing of both the nameplate and the graphics.
- Andy Davies for the photo.
- Wim De Waegeneer for his cartoon.
- Laura Donohue for proof-reading.
- Terry Donohue and Richard Mitchell (The Underground Grammarian) for supplying some of the graphics.
- Anne Edwards for her cartoon.
- Joan Green for the Current Awareness List
- Dee Rawsthorne for her article.
- Keith Roberts for poems and quotes.
- Black Rot for the crossword.

# Project Summaries

*From Sue Albini...*

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

The accumulation of synaptonemal complex (SC) preparations continues. It is still a slow and tedious process, so modifications and improvements to the basic method are being actively pursued. A wide range of stages, from early zygotene to late pachytene, have been identified in the electron microscope. This enables examination of the mechanics of homologous chromosome pairing. In *Arabidopsis*, despite the small size of the chromosomes, there are still many sites of pairing initiation within the genome. This is a feature common to all the plant systems so far investigated. One area of interest in the lab. at Birmingham is the relationship between SC complement length and amount of genomic DNA.

"This indicates a much lower ratio of DNA amount/SC length than in other plants..."

It has been proposed that this is a linear relationship in higher plants, so an increase in the amount of DNA produces a unit increase in SC length (Anderson *et al*, 1985, Exp. Cell Res. 156, 367-378). However, this does not seem to be the case. Small genomes have, proportionally, a much longer SC complement length than large genomes. The results from *Arabidopsis* are an interesting addition to these data. In the nuclei measured so far, the SC complement length is about 200 µm. This indicates a much lower ratio of DNA amount/SC length than in other plant species, e.g., *Allium cepa* has a 1C DNA amount of 33.5 pg and the mean pachytene SC complement length is 753 µm. This implies that there is variation between species in the way the genome is organised during chromosome pairing. This might be related to the ratio of repetitive/non-repetitive DNA as well as total DNA amount. The first results of experi-ments to label SCs with DNA probes, by *in situ* hybridisation, indicate that this line of research will be very fruitful. SCs of rye have been labelled with a ribosomal DNA probe. The method for *in situ* hybridisation of SCs with DNA probes is currently being developed and refined.

S.M.Albini, G.H.Jones & J.S.Parker; School of Biological Sciences, University of Birmingham, P.O. Box 363, Edgebaston, Birmingham B15 2TT.
ALBINISM@UK.AC.BHAM.IBM3090

*From Ken Buck...*

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

Tobacco leaf discs have been transformed with the OR1 vector described in the last newsletter with and without an origin of DNA replication provided by tomato golden mosaic virus DNA A. Calli resistant to kanamycin have been selected and plated on a medium containing hygromycin. Calli containing the TGMV replicon were able to grow on the hygromycin medium suggesting that intramolecular recombination had occurred, as predicted, to generate a functional hygromycin phosphotransferase gene. DNA analysis is in progress to determine if a circular replicon has been generated.

Calli containing the OR1 vector, but no replicon, survived longer on medium containing hygromycin than did control calli transformed by pBin19, but eventually died. It is possible that a functional hygromycin phosphotrans-ferase gene was generated, but eventually lost during multiplication of the callus cells, because of its inability to replicate.

Construction of a second OR1 vector designed to regenerate a functional neomycin phosphotransferase gene by intramolecular recombination is in progress.

T.D. Jones & K.W. Buck; Dept. of Biology, Imperial College of Science, Technology and Medicine, London SW7 2BB.

*From David Coates...*

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

### Sequencing the plant plasma membrane Ca pump (PMCP).

Joy Boyce and David Evans.

The last three months has seen further confirmation that the inserts detected by immunoscreening our λgt11 expression library are representative of the PMCP. In addition to expressing protein recognised by an antibody monospecific to the PMCP, the cDNA inserts could be primed for sequencing by synthetic oligo-nucleotides representing the highly

"Sequencing the inserts is now at an advanced stage..."

conserved hinge region of the P-type ion translocating ATPase, based on computer prediction and micro-peptide sequencing the plant protein. However, it seems unlikely that they include cDNA encoding the calmodulin binding region of the protein as synthetic oligonucleotides to this region do not bind the inserts. The fact that the expressed protein is recognised by the antibody is, however, indicative of the fact that some part of this region is likely to be encoded, suggesting that the cDNA inserts run from the hinge towards the C-terminus, encoding several trans-membrane helices and some part of the cytoplasmic C0-terminus. Sequencing the inserts is now at an advanced stage and will be completed by Christmas. We're hoping for good homology with the other P-type Ca pumps!

### Post-translational regulation.

Per Askerlund & David Evans.

We have been looking at pure plasma membrane as a better source of the PMCP for activity and reconstitut-ion studies. We have purchased a "Liposomat" liposome production system in order to make well characterised vesicles and (in association with D.T. Cooke, Long Ashton) have begun to analyse the »›

phospholipid environment of the PMCP *in vivo*.

This is the beginning of a major attempt to investigate the role of phospholipids in regulating PMCP activity.

### Cloning the *Arabidopsis* plasma membrane Ca pump.

Janice Coates & David Coates.

We have used four oligonucleotide as primers for PCR amplification of Ca pump sequences from genomic DNA. One pair are based on the *Zea* peptide fragments identified by the antiserum to erythrocyte Ca pump, the other based on homology studies of P-type ATPases and calmodulin binding regions (see above). All permitted combinations of primers amplify fragments of the right sort of size (900 to 1200 bp), though the nested PCR experiments have still to be done. In anticipation, DC has constructed a representative genomic library of *Arabidopsis thaliana* in λ DASH II. The library is based on a SauIIIA partial digest cloned into the BamHI site of the vector, with an average insert size of 17Kb. We hope soon to have identified positive clones using the PCR product(s) as probes - more at Xmas.

Drs D. Evans, D. Coates, B.S. Cox, with Drs J. Boyce, J. Coates & P. Askerlund; Plant Sciences Oxford and Pure & Applied Biology, Leeds.
PAB6DC@UK.AC.LEEDS.BIO.VAX

*From George Coupland...*

### A two-component transposon tagging system in *Arabidopsis*.

The autonomous *Ac* element was reported to transpose at very low frequencies in *Arabidopsis* (Schmidt and Willmitzer (1989) MGG 220; 17-24). As the single *Ac* transcript is expressed at very low levels, we decided to fuse the *Ac* ORF to stronger promoters, and to test the efficiency with which the fusions drive transposition of a second *Ac* derivative which was previously mutated *in vitro* such that it no longer contains an intact ORF. This second element, called *Ds*, is inserted within the untranslated leader region of a streptomycin resistance gene so that excisions of the element can be detected phenotypically.

So far we have studied in most detail fusions of the octopine synthase promoter, the nopaline synthase promoter, the CaMV35S promoter and the *Ac* promoter (30–440 bp). We have transformed *Arabidopsis* roots with these constructs and regenerated plants. For each construct we have identified five independent transformants (except for pOCS for which we have four) which are single locus insertions. We have followed the segregation pattern of kanamycin resistance for all 19 transformants and for each one have identified plants homozygous for the T-DNA. In parallel, a plant homozygous for the *Ds* construction was identified.

> '60% of the seedlings...have inherited germinal excision events...'

We have now crossed the plants containing the *Ac* ORF fusions to those containing *Ds*. The F1 seeds were then sown on streptomycin. During the development of these plants excisions of the *Ds* were expected to occur. Clones of green, streptomycin resistant cells were indeed detected on the cotyledons of the F1 plants in crosses involving the CaMV35S, OCS and *Ac* promoter fusions. These clones are assumed to be the result of somatic excision of the *Ds* element. No excisions were detected when the nopaline synthase promoter fusion was used. Interesting differences in the pattern of somatic excisions were detected. For example, in the crosses involving the p*Ac*-*Ac*ORF and the pOCS-*Ac*ORF fusions not all of the progeny showed excision although they were genetically identical. Of 185 F1 seedlings tested on streptomycin after crossing plants containing p*Ac*-ORF to those carrying Spt::*Ds* only 44 showed somatic excision, and the excision frequency varied dramatically between individuals. Similarly, for the pOCS-*Ac*ORF fusion only 55 of 91 seedlings tested showed variegation, although in this case the patterns were rather similar between spotted individuals. The lack of penetrance detected with these two fusions was very different to our results with the CaMV35S-*Ac*ORF fusion, where 100% of the seedlings expected to show excision were indeed variegated. Moreover, the spotting frequency was much higher when the CaMV35S promoter was used.

The variegated F1 progeny were then grown to maturity and the F2 seeds collected. These seeds were then sown on streptomycin-containing medium. The differences in excision frequencies detected in the F2 is astounding. The pOCS-*Ac*ORF fusion produced no full green individuals which had inherited a germinal excision event. Moreover, variegated seedlings were very rare and made up less than 1% of F2 seedlings tested. We infer from this that pOCS is being inactivated during the F1 generation. The F2 progeny derived from crossing plants carrying the pCaMV35S-*Ac*ORF fusion to those harbouring Spt::*Ds* are very different from those described for the pOCS-*Ac*ORF. 60% of the seedlings in some of the F2 families derived from this cross appeared to have inherited germinal excision events: the theoretical maximum is 75%. There are also large numbers of variegated progeny in some families. Our present feeling, however, is that the frequency of somatic spotting in these F2 individuals is lower than that seen in the F1. The CaMV35S promoter might therefore be undergoing inactivation similar to that seen for the OCS promoter, but its effect is less severe, perhaps because the normal transcriptional level of the CaMV35S promoter is so much higher.

Are the full-green individuals we see in the F2 generation real germinal transposition events or are they just seedlings in which somatic transposition is occurring at very high levels? Our promoter fusions to the *Ac* ORF are on a T-DNA which also contains a GUS gene. If the full greens are germinals then 25% of them should be GUS negative as the T-DNA containing the *Ac*-ORF ➳

need not segregate with the the one carrying the $SPT^R$ gene. However, if they are somatic events they should all be $GUS^+$, as the *Ac*-ORF must be expressed in the plant containing the SPT::*Ds*. We have tested 330 full-green $F_2$ plants derived from 22 $F_1$ variegated plants and 100 were $GUS^-$ while 230 were $GUS^+$. This proportion is close to the 3 $GUS^+$:1 $GUS^-$ expected if all the full greens tested were indeed germinal excision events.

Many of these $SPT^R$ $GUS^-$ individuals should contain a *Ds* at a new location. This new position might be within a gene. If this is the case then 25% of the individuals in an $F_3$ family will be homozygous for the insertion, and might show a mutant phenotype. We are presently growing the $F_3$ progeny of 42 of our $SPT^R$ $GUS^-$ plants and will screen them for mutant phenotypes.

What proportion of $F_3$ families might be expected to segregate mutant phenotypes? This is difficult to answer as we don't know the density with which genes occur on the *Arabidopsis* chromosomes, or how many of them will produce a mutant phenotype if inactivated. However, if we can extrapolate from the frequency with which the insertion of T-DNAs cause mutant phenotypes in *Arabidopsis*, we would estimate that 1-10% of our $F_3$ families will show mutant phenotypes. An extra complication is that *Ds* will probably transpose to genetically linked sites in *Arabidopsis*, as this has been shown to occur in maize and tobacco. To isolate specific mutants with *Ds* (so called targeted tagging) therefore, it will be important to know where our *Ds* containing T-DNA is inserted. We have isolated *Arabidopsis* DNA flanking this T-DNA by inverse polymerase chain reaction and will try to map it by hybridising it to the YAC library or by RFLP mapping. We have also isolated another 11 *Ds* containing lines and intend to map them also.

J. Swinburne, LL Balcells, K. Ingle, C. Recknagel, S. Scofield, J. Jones & G. Coupland; JI Centre for Plant Science Research, Cambridge Laboratory and Sainsbury Laboratory.

☆*A star, as promised, for George for not only producing such a thorough report, but for also getting it in a week early and on disc. -- ACM.*

*From Simon Covey...*

## Isolation of *Arabidopsis* genes involved in cauliflower mosaic virus pathogenesis.

A requirement of isolating host plant genes involved viral pathogenesis is an efficient means of screening for plant mutants that show altered responses following inoculation with virus. In principle, screening for plant variants to CaMV infection is fairly straightforward: plants are inoculated with virus and, after an intervening period, the severity and character either of local inoculation lesions (visible after 6-8 days), or of systemic symptoms (developing after 10-15 days) is scored. Plants with symptoms of enhanced or attenuated severity, or those that are asymptomatic are potentially of interest. So far, from screenings of 25 *Arabidopsis* ecotypes infected with our severe type CaMV strain Cabb B-JI, we have not identified any significant variations in symptom severity worthy of further study. A problem we sometimes encounter is variability in response of plants within an ecotype due to the relatively advanced age at which it is necessary to inoculate. The source variability is thus presumably physiological. It is very difficult to successfully inoculate tiny *Arabidopsis* seedlings without severely damaging them using conventional mechanical inoculation techniques.

"Experiments suggest that 2,4-D treatment somehow enhances the uptake of virus..."

However, a rather intriguing observation of ours arising from a separate series of experiments might lead to a solution to inoculation problems. As part of a programme to establish various types of tissue cultures from *Arabidopsis*, we have been germinating sterile *Arabidopsis* seeds in different culture media including one containing the auxin 2,4-D. Under these conditions, plants germinate as normal and produce cotyledons, leaves and roots. After about two weeks in culture, the plants in media with 2,4-D are more stunted than controls and eventually callus tissue proliferates. In comparing seed from different sources, we discovered that batches of seed obtained from plants infected with CaMV germinated in 2,4-D medium as normal, but by about 10 days in culture the green parts of all of the plants had become completely chlorotic. Although CaMV is considered not to be seed transmitted, there are reports in the literature of the presence of CaMV virions in *Arabidopsis* seed coats.

To determine whether the effect was due to CaMV infection, we incubated seed obtained from healthy plants with purified CaMV virions and found that seedlings became chlorotic when cultured in the presence, but not in the absence, of 2,4-D. These experiments suggest that 2,4-D treatment somehow enhances the uptake of virus into imbibing seeds to establish an early infection. However, we have not yet formally demonstrated that the tiny seedlings are infected and not suffering from some toxic effect of the virus preparation. We are repeating the 'inoculations' with infectious and non-infectious virus DNA preparations to clarify this point.

If the phenomenon we observe is a result of virus replication, then screening of mutant stocks for resistant plants would be enhanced. In principle, millions of seeds at a time could be screened for variants.

Andrew Bannister, Charles Greif, Simon Covey & Andy Maule; John Innes Institute, Norwich.
COVEY@UK.AC.AFRC.JII

*From Caroline Dean...*

### Transposon tagging.

Caroline Dean, Emily Lawson, Tania Page & Ian Bancroft

We are working our way through the analysis of the Landsberg *erecta* transformants carrying the different versions of the autonomous *Ac* element. We have followed the transposition frequency of *Ac* in single and multi-copy transformants through four generations. We have also backcrossed all the multi-locus transformants carrying the NaeI deleted *Ac* element to Landsberg *erecta* to obtain non-segregating lines for future analysis. Southern blots of these plants show that the majority of them contain a single ➤

T-DNA at each locus. Progeny of plants picked to contain a transposed *Ac* are still being screened for segregating mutations. Several putative mutants are being pursued, but a low level of somaclonal variation in the transformants can often get us all excited unnecessarily.

Ian Bancroft has been working with a two element transposon system using a stable NaeI deleted *Ac* element (linked to a GUS marker) and a *Ds* element carrying a 35S-hyg fusion. The *Ds* vector (0282) also has a 30bp sequence containing three rare cutter sites positioned in the T-DNA and also in the *Ds* element. This will allow him to compare the physical transposition distance of the *Ds* elements on pulse field gels with the genetic distance as measured by the recombination between the streptomycin (our excision marker) and hygromycin markers. The germinal excision frequency using the NaeI deleted stable *Ac* was between 1 and 5% depending on the transformant (all of which carried a single T-DNA locus) and he is currently checking on the independence of these germinal excision events.

"Germinal excision frequency using the NaeI deleted stable *Ac* was between 1 and 5%..."

Anuj Bhatt currently finishing his Ph.D thesis at the University of Glasgow will be starting to work on this project at the beginning of December. I hope he doesn't mind lots of screening for mutant phenotypes!

### The *Arabidopsis* genome project.

Gerda Cnops & Renate Schmidt

Improvement of the hybridisation conditions have led to much clearer results in the colony hybridisation experiments. This has allowed us to pull out YAC clones for almost all RFLP markers located on the top halves of chromosomes 4 and 5. Some RFLP markers did not light up clones in the library of the Columbia ecotype. For these, a screen of a second library (*ca.* 2100 clones, ecotype Landsberg *erecta*, provided by E. Grill and C. Somerville) turned out to be useful.

Most of the YAC clones that we have identified so far have been sized using PFGE (size range of clones: 75-265kb). Furthermore, we were able to screen the libraries with chloroplast DNA of *Arabidopsis* (gift of A. Brennicke and W. Schuster). In both libraries we found approximately 100 clones hybridising to chloroplast DNA.

We are about to start our walking experiments on chromosome 4 in the region around *fca* (RFLP markers: 518, 210, 455, 580, 326, 226)

### The mechanism of vernalization.

John Chandler

Work is continuing on the physiology of flowering in the late flowering mutants by treating plants grown *in vitro* with different compounds in an attempt to find a function for the late flowering genes.

We have been screening an EMS-mutagenised population of the *fca* mutation (one of the late flowering mutants isolated by Maarten Koornneef) to isolate individuals that are no longer responsive to vernalization. Individuals flowering at the same time as non-vernalized *fca* have been backcrossed to Landsberg *erecta*. We are searching for secondary mutations which are not themselves late flowering, but which are mutations in the perception and transduction of the cold temperature signal. We have also identified putative suppressor mutants of the *fca* mutant that flower at the same time as wild type Landsberg *erecta* without a vernalization treatment.

### Genetic and physiological analysis of vernalisation requirement in the winter annual Stockholm.

Jonathan Clarke.

The mapping of two loci (*Fri* and *kry*) conferring a vernalization requirement in the ecotype Stockholm continues. We have $F_3$ families segregating for flowering times from which we are currently preparing DNA. We will search for RFLP markers segregating with the loci. Initial problems with Southern analysis with Meyerowitz's RFLP probes have finally been overcome. The lines carrying *Fri* and *kry* (in Li5) obtained from Prof. Napp-Zinn in Cologne have been crossed to Landsberg *erecta* and then backcrossed to Landsberg *erecta* five times. We want to obtain the *Fri* and *kry* loci in the Landsberg *erecta* background so that they will be isogenic with the other late flowering mutants. We hope to use the techniques of 'Genomic Selection' to ensure that the lines are isogenic. After screening a number of possible *in vitro* culture medium I have finally settled on one recommended by Lother Willmtzar's lab., *AM*:

1/2 Strength Murashige and Skoog Macro- and Micro-Salts:

| | |
|---|---|
| 219.5 $mgl^{-1}$ | $CaCl_2.H_2O$ |
| 0.0125 | $CoCl_2.6H_2O$ |
| 0.0125 | $CuSO_4.5H_2O$ |
| 18.35 | Fe Na EDTA |
| 3.1 | $H_3BO_3$ |
| 85 | $KH_2PO_4$ |
| 0.415 | KI |
| 950 | $KNO_3$ |
| 185.3 | $MgSo_4.7H_2O$ |
| 11.15 | $MnSO_4.4H_2O$ |
| 0.125 | $Na_2Mo_4.2H_2O$ |
| 825 | $NH_4NO_3$ |
| 4.3 | $ZnSO_4.7H_2O$ |

• Murashige and Skoog Plant Salt Mixture can be obtained pre-made from Flow labs.

1/2 Strength Gamborg's B5 Vitamins:

| | |
|---|---|
| 50 $mgl^{-1}$ | Inositol |
| 0.5 | Nicotinic acid |
| 5 | Thiamine HCl |
| 0.5 | Pyridoxine Hcl |

Adjusted to pH 5.7

Supplemented wit:h

1% Sucrose (Optional)

and 0.8% Difco Bacto agar

10 ml of medium are placed in pre-autoclaved 25x150 mm culture tubes (Sigma, C5916) with caps (Closure Sigma, C5791) and supported on autoclavable racks (Culture tube rack Sigma, C5541). Plants are grown at a light intensity of 100 $\mu Em^{-2}s^{-1}$ at $20^0C$ with a photoperiod of 16 hours. It was found that darkening the roots with black card or plastic improved root growth. This medium has now been used successfully in Physiological experiments by John Chandler.

NB. If anyone has had experience with methods of 'Genomic Selection' in *Arabidopsis* I would be very interested to hear from them.

C. Dean *et al.*; Norwich.
ARABIDOPSIS@UK.AC.AFRC.JII

# Project Summaries

*From John Doonan...*

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

Our aim is to identify plant homologs of fungal cell cycle genes and determine if plant genes are involved in nuclear structure and division in a similar manner to their fungal counterparts.

Using information derived from fungal gene sequences for PCR oligos or using the gene as a probe, we are about to commence the search for the *nimA* protein kinase, a positive regulator of the G2/M transition in *Aspergillus*.

We have recently demonstrated that antibodies against the yeast nuclear pore protein, NSP-1, recognise the plant nuclear pore. Anti-NSP-1 stains the plant nuclear envelope during interphase (by indirect immunofluorescence). Immunogold demonstrates that this is due to the presence of the cross-reacting antigen at the nuclear pore. In co-operation with Beven & Shaw, we have show by immunoblotting that the antigen is a component of the plant nuclear detergent-resistant skeleton. Cloning of the plant homolog will be of some interest, since it would appear to resemble the yeast homolog somewhat more closely in molecular weight (100kD) than does the animal homolog (62kD).

John Doonan; Dept. Cell Biology, John Innes Institute, Colney Lane, Norwich, NR4 7UH.

*From Gary Foster...*

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

Pollen specific promoters are now into plants with GUS, antisense and sense of our pollen specific genes.

"We are in the process of constructing a number of cDNA libraries."

We have not much more to report at this stage, but we anticipate that we will compile the longest and most outstanding report to date in the next newsletter (just to keep the ACM happy).

One other small point of interest is that we, i.e., Rachael Hodge and Adrienne Hair, are currently in the process of constructing a number of cDNA libraries to the following RNA populations:

a) small *Arabidopsis thaliana* buds
b) *Brassica napus* buds (lesss than 1mm)
c) archesporial cells from *Brassica napus* anthers.

We will report the status of these libraries in our next report, but would be interested to hear from anyone also interested in these libraries.

Dr. Gary Foster, Dr. Rod Scott , Dr. John Draper, Mr. Mike Roberts, & Mr. Rob Blundell; Botany Department, University of Leicester, LEI 7RH .
Tel: 0533-523393; FAX: 0533-471001

*From Ian Furner...*

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

Karen Sweet and I have been continuing work on seedling mutants that affect the stem apex and have also started work on generating marked somatic sectors to "fate map" the apex. We have taken two approaches:

1) EMS mutagenesis of seeds heterozygous for albino mutations and;
2) Introduction of a 35S-*Ac*-RolC construct kindly provided by Angelo Spena. This construct should generate yellow sectors on excision of *Ac*. [Spena et al. (1989) The Plant Cell 1, 1157-1164]. The first approach seems to work and the second does not. We have only observed one sector in several hundred transgenic plants containing this construct. Paradoxically the germinal exision rate in *some* of these lines seem high -- up to 10%. (I do not understand this).

Paul Davison has made a cDNA library derived from *in vitro* produced shoot apices in ZAP. The average insert size is rather small and we would not recommend its general use. It is probably good enough for getting the initial probes for our work by differential screening with cDNA from other organs/tissues.

Karen Sweet has decided to leave the project and so work on the genetics and fate mapping will be slowed down while I seek a replacement. Ottoline Leyser has finished her Doctorate and gone to Mark Estelles laboratory in Bloomington. Congratulations Ottoline, now about writing up those papers....Two new *Arabidopsis* postgraduate students have joined the laboratory.

More from Ian in the accompanying protocols

Ian Furner; Dept. of Genetics, University of Cambridge, Downing Street, Cambridge, Tel. 0223-333959.

*From Nic Harberd...*

## An attempt to clone the gal locus by phenol-enhanced DNA re-association.

Further to our attempts at deletion cloning we are setting up to use the genomic subtraction method (see Strauss protocol in previous issue). I know several other laboratories are (planning on) doing the same and I think it would be useful to keep in touch.

Mary Holdsworth and Nicholas Harberd; Cambridge Lab., Norwich.

*From Nick Harris...*

## Development of the silique of *Arabidopsis*.

Jacqui Spence has continued with a morphological and cytological characterisation of silique development in wild-type and *clv* mutants using a range of histo- and cyto-chemical techniques. These include some immunological work with a few of JI's JIMs (*John Innes monoclonal antibodies --ACM*). »

She has been paying parting particular attention to the development of the vasculature, arrangement of septa, and differentiation of endocarp layer(s). All of which have important roles in seed and fruit development and the dehiscence mechanism. We are screening EMS-treated seeds and have selected a number of promising mutants, which are currently being taken to M2 fruiting.

Nick Harris & Phil Gates; Dept. of Biological Sciences, University of Durham.

*From Pat Heslop-Harrison...*

## The chromosome structure and genome organisation of *Arabidopsis* in reconstruction of nuclei.

Using non-radioactive *in situ* hybridization we are connecting the genetic linkage map and RFLP maps with the karyotype of *Arabidopis thaliana*. Our photographs show the use of a wheat ribosomal DNA probe (pTa71; Gerlach & Bedbrook NAR 7, 1969) which localizes the rRNA gene clusters on the chromosomes.

The probe was labelled with biotin or digoxigenin before *in situ* hybridization to metaphase chromosomes and interphase nuclei of root tip cells. Detection of hybridization sites with fluorescein conjugated molecules (avidin-FITC is shown, upper) or enzyme linked molecules (e.g. avidin-horseradish peroxidase, with enzymic precipitation of DAB, diaminobenzidine) confirms the presence of two pairs of nucleolar organising chromosomes. DAPI staining (shown, lower) of DNA following *in situ* hybridization allowed these to be identified as chromosomes number 2 and 4. As well as using some of the Meyerowitz RFLP probes to localise them along the chromosomes, we are also investigating the expression of the rDNA genes at interphase using electron microscope images of spread chromosome preparations, such as those shown, after transfer to EM grids.

Current problems include high background, particularly with single copy, short, probes, and the low number of metaphases in each root tip. We have strategies to overcome the first problem, but not the second. Does anybody out there have a high-mitotic index mutant, or perhaps a rapidly dividing suspension culture?

J.S. Heslop-Harrison and J. Maluszynska, Karyobiology Group, J.I. Centre for Plant Science Research.

*From Eric Holub...*

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

Significant progress has been made in studying the response of *Arabidopsis* to the obligate fungal parasites *Albugo candida* and *Peronospora parasitica*. We are currently maintaining two isolates of *P. parasitica* that can now be differentiated using host lines collected from wild U.K. populations. The lines include types that are susceptible to both isolates or resistant to both, and resistant to one isolate but susceptible to the other (in both combinations). Of particular interest is the observation that Landsberg *erecta* and Columbia are resistant to both isolates. We have corresponded with Alan Slusarenko, co-author of a recent paper describing variation in *Arabidopsis* for response to a Swiss isolate of *P. parasitica* (The Plant Cell 2:437-445). The intention is to develop a cooperative effort with him to study the number and chromosomal location of genes for resistance to this fungus.

"We would be delighted to receive samples of seed collected from any geographic source."

Thus far, *Arabidopsis* resistant to *A. candida* appears to occur less frequently. Columbia, Landsberg *erecta*, ecotypes from Cape Verde Island and Poland, and all of the Kent collections tested so far have been susceptible to an isolate of the fungus collected from East Malling. However, progeny from a plant found in the Lake District proved to be resistant to this isolate, whereas other plants from the same population were found to be naturally infected, possibly by a different pathotype of the fungus. We are currently comparing the virulence of the two isolates of *A. candida* on a wider range of host ecotypes.

In the previous newsletter, we reported the isolation of a *Phoma spp.* from a field-grown plant of *Arabidopsis*. The fungus has formed pycnidia on rosette leaves when *Arabidopsis* was inoculated by spraying plants with conidia without intentional wounding. This evidence of fungal development has been seen infrequently among inoculated plants and affected leaves have always been the oldest of a rosette or ones infected by *A.candida*. Nevertheless, the isolate has been found to be virulent to *Brassica napus* and *B. rapa*, and plants of these species differing in susceptibility to the fungus have been selected. We plan to attempt sexual crossing of the isolate from *Arabidopsis* with known brassica isolates of *Leptosphaeria maculans* (sexual form of *P. lingam* and causal agent of blackleg of brassicas), and will continue attempts to select for higher host susceptibility among collections of *Arabidopsis*. We would be delighted to receive samples of seed collected from any geographic source, but especially from Britain for testing responses to all three fungi.

Eric Holub and Ian Crute; IHR, ➳

East Malling, Maidstone, Kent ME19 6BJ.
HOLUBE@UK.AC.AFRC.EMRSA

*From Gareth Jenkins...*

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

There is litle to add to our last contribution to the Newsletter. Our main objective is to produce transgenic *Arabidopsis* expressing various chimaeric constructs which we can subsequently use to screen for photoregulatory mutants. We achieved transformation early on and are now trying to produce and express the various constructs that we need. At present we are working with several different promoters and are investigating an alternative reporter gene to luciferase. In addition, we have started a conventional screen for mutants altered in photoregulation.

Our studies of the *hy-4* mutant, which is deficient in the blue light control of hypocotyl extension, have progressed a little further. It is clearly altered in several additional ways in its growth in blue light, for example in petiole length, and we are attempting to quantify these differences. We are trying to establish whether it is altered in responses only in blue light, and if so, in all or only some responses.

Gareth Jenkins, Karen Deeney and Jennie Jackson (and also Nigel Urwin), Departments of Biochemistry & Botany, University of Glasgow.

*From Kerrie Jones...*

## Ammonium toxicity in *Arabidopsis*.

In the last report we described our efforts towards cloning an *Arabidopsis* glutamate dehydrogenase (GDH) gene(s) using a nested PCR approach. Unique products were amplified from the genomic DNA of several organisms, including *Arabidopsis*, using our "universal GDH primers". These primers are based on alignments of all known GDH sequences. The PCR products have now been confirmed as fragments of gdh genes in two ways. First, by Southern blotting using the *Aspergillus nidulans* gdh gene as a probe. Second, by direct sequencing of the products. As expected, all fragments share significant homology with known GDH sequences and that from *Dactylium dendroides* has an intron at the same location as in other fungal gdhs.

Screening of an *Arabidopsis* genomic library (from David Coates) is now underway using the *Arabidopsis* GDH fragment as a hybidisation probe. We are also currently screening M2 seeds (Lehle Seeds) to look for mutants which display tolerance to ammonium.

Kerrie Jones (1,2), Mike McPherson(1) & Andy Cuming (2); 1 Dept. of Biochemistry and Molecular Biology, 2 Dept. of Genetics, Leeds University.
KERRIE@UK.AC.LEEDS.BIO.VAX

*From Peter Jordan...*

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

PCR-amplified fragments of both the hemC and hemE genes from an *Arabidopsis* gene library are being cloned into M13 for sequencing purposes. The hemB gene is currently being amplified by a similar approach. We have identified suitable regions of the hemL gene in order to prepare oligonucleotides for use with PCR. We are also refining direct sequencing methods for PCR products and hope this will make it unnecessary to clone into M13.

The work on isolating additional enzymes has been delayed by the high temperatures in the summer killing the plants. We should be able to continue with this part of the programme shortly. We are also about to commence probing a cDNA library and hope to have both gene and cDNA sequences for at least three genes of the chlorophyll pathway.

Prof. 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*.

We have now analyzed *ca.* 50 *Arabidopsis* primary transformants for GUS activity from native promoter-GUS fusions following transformation with a promoterless GUS construct. Some 28% of these transformants exhibit GUS activity in aerial tissues. Similarly, 21% of 231 tobacco transformants have now been shown to exhibit GUS activity in leaves, although at least 45% of all tobacco transformants show GUS expression in leaf or root.

To date, lack of seed set in *Arabidopsis* transformants has proved to be a problem. However, this has now been largely resolved and a significant proportion of transformants are now setting fertile seed. T1 seed has been collected and segregation analysis and the analysis of GUS fusion gene expression can now go ahead. GUS expression throughout the development of diverse organs will be assayed. Segregation analysis will be supplemented with Southern hybridization analysis to determine the copy numbers of the GUS inserts.

Seed stocks of transformed lines will be bulked up to allow growth of populations of plants in soil. Screening for developmental and/or phenotypic mutants can then proceed as these may not be readily apparent in plants grown in constrained sterile culture conditions.

Keith Lindsey, Mike Clarke, Jennifer Topping & Wenbin Wei; Leicester Biocentre, University of Leicester.
DRL @UK.AC.LEICESTER

*From Andy Maule...*

## Identification and expoitation of the interaction between a protein and host factors which control virus spread.

We are continuing to gather the tools together. The *Arabidopsis* primary transformants with CaMV ➤

gene I look promising with 19 candidates yielding 12 potential single copy insertions and only three escapes – thanks to Annette and Caroline. We hope we can learn from Jon Clarke's experiences and tackle the genomics when we have enough material. After a single attempt, the microprojectile bombardment of Columbia seedlings with cloned CaMV DNA did not give an infection, except with fungi and bacteria! Success next time!? The answer to the problem of instability with CaMV multimeric constructs will be revealed at Christmas.

There once was a crucifer virus,
To spread cell-to-cell was its bias,
It duped the host
Into helping it most,
How, when and with what it won't show us --- yet.

Andy Maule, Carole Harker & Genevieve Boudazin, John Innes Institute, Norwich.

*From Keith Mitchelson & Deborah Silcock...*

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

We are still labouring through the screening of the YAC genomic library and *Arabidopsis* ecotypes for informative repetitive elements. We have explored several protocols to transfer YAC DNA efficiently to nylon membranes for hybridisation. U.V. nicking of YACs in agarose works best (Fotodyne 300nm) with only a small increase in hybridisation signal for nicking times between 30 and 500 seconds. Concerns that mixing of the clones to facilitate screening of the library might result in under-representation of individual clones have not yet been established.

"Does anyone have a protocol which gives DNA fragments consistently larger than 100kb?"

Although work is in progress to compare the efficiency of screening from pooled cultures containing up to eight clones, with eight clones grown up individually which are subsequently pooled. The grind continues....

Does anyone have the protocol for an *Arabidopsis* DNA preparation which gives DNA fragments consistently larger than 100kb? The CTAB and other rapid procedures seem to be variable.

Deborah Silcock & Keith Mitchelson; Dept. of Molecular and Cell Biology, University of Aberdeen.

*From Jim Murray...*

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

The suspension cultures for the cDNA library continue to grow well, if slowly, on Murashige and Skoog medium supplemented with 25M 2,4-D and with the sucrose reduced to 10g/l. Cultures are re-fed with fresh media every 5-7 days and subbed at 4-6 weeks. The size of individual calli in the suspension has dropped steadily with subculturing to become more uniform, with calli 1mm diameter. I hope to prepare mRNA from culture samples soon now that we have received two of the expression vectors that we hope to modify for our library. We have had some success in demonstrating the use of redundant PCR as an alternative to complementation for cloning conserved genes. Using primers directed against the conserved cdc2 gene we have amplified fragments from both *Arabidopsis* and *Chlamydomonas* using genomic DNA from each plant as a template. The size of the amplified fragments (~390bp) is identical to that of the amplification product from control DNA (*Xenopus*) and agrees with the size prediction based on the the conserved amino acid sequence data for known cdc2 homologues. This result also suggests that there are no intervening sequences at least in these regions in the plant genomic DNA. We have cloned the fragments from both plant species are in the process of sequencing the inserts. Now that we know it works we hope to expand on this part of the project by designing more oligo primers directed against genes other than cdc2 which display significant amino acid sequence conservation.

Jeremy Carmichael & Jim Murray; Institute of Biotechnology, University of Cambridge. Tel 0223 334754. JAHM@UK.AC.CAMBRIDGE.PHOENIX

*From Steven Neill...*

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

No dramatic advances since the last report three months ago! However, we have made *some* progress. The time-course of ABA synthesis following the imposition of water stress has been determined. ABA concentrations in shoot tissue increase two-fold within thirty minutes after the loss of turgor, have increased ten fold after eight hours and are still increasing after twelve hours. mRNA extracted from leaf tissue at increasing times after the impositionof water stress is being analysed by translation *in vitro*.

"We have a...cDNA library from wilted leaves."

We have had considerable difficulty in growing the ABA-deficient wilty mutants in sufficient amounts to get mRNA. However, we have finally got our high-humidity growth cabinet and are now raising plants for mRNA extraction.

In conjunction with Mike Bulman, a SERC-supported RA, we have made what appears to be a reasonable cDNA library from wilted leaves. We made this library using the Stratagene "Unizap" cloning kit, after some problems with a kit from Clontech. We are now at the stage where we can begin to screen this library with probes representing mutant and wilted wild type tissue.

Finally, we are thinking of using PCR-amplification of cDNA to get around the problem of a lack of mutant tissue. It may then also be possible to make a subtractive cDNA library to increase our chances of finding stress related sequences.

Steve Neill & Jackie Williams, Bristol Polytechnic, Bristol.

*From Helen North...*

## Cell cycle control genes in *Arabidopsis*.

Despite problems with BRL incompetent cells, some *S. pombe* genomic DNA has been obtained and this is now to be used to test our competency at transforming and complementing two *S. pombe* cytokinesis mutants. RNA has/is being prepared from carrot protoplasts and cauliflower for construction of cDNA libraries in *S. pombe* expression vectors.

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

*From Jane Parker...*

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

Work continues on the interaction of various *A. thaliana* ecotypes with strains of the bacterial pathogen *Xanthomonas campestris* pathovar *campestris* (and at the same time searching for plants which are resistant to scarab fly infestation). Conditions for plant growth and *X.c.c.* infection have been standardised and we are now testing the segregation of resistance and susceptibility to *X.c.c.* strain 1067 in the $F_3$ progeny of $F_2$ individuals derived from a Columbia x Landsberg *erecta* cross. The first set of results showed a clear phenotype which corresponded to the $F_2$ parent in about one third of the plants. The remaining plants are being tested again. It is clear that strain 1067 is a weaker pathogen overall than 8004 and symptom development on susceptible plants is less distinct and slower. It is therefore necessary to score symptoms over a period of days to be sure of the reaction phenotype. We have, however, convinced ourselves that resistance in Columbia arises from a positive interaction with 1067. Chris Barber has been analysing a 25Kb DNA fragment derived from 1067 which, when transconjugated into 8004, confers the 1067 phenotype on all *A.thaliana* ecotypes tested so far. It doesn't affect extracellular enzyme production and causes 8004-like symptoms on turnip plants. We are now trying to dissect out the putative avirulence gene (or genes) from the DNA by subcloning and Tn-5 mutagenesis. We also want to confirm preliminary data which suggest that the dominant locus in Columbia which determines resistance to 1067 interacts in the same way with the avirulence gene.

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

*From Jo Putterill...*

## Isolation of the flowering-time gene *fg*.

The flowering-time gene *fg* is located on the upper arm of chromosome 5 at 16.4cM. We have initiated a chromosome walk from the tt-4 marker which is 2cM (approximately 300kb) distal to *fg* and encodes the enzyme chalcone synthase. The Columbia DNA YAC library, from Chris Somerville's lab, was screened with RFLP markers that are in the vicinity of *fg*. This screen (carried out by Renate Schmidt) identified a number of positively hybridising clones. Four YAC clones hybridised to chalcone synthase. Three of these clones also hybridised to the cosmid marker 6833. One clone hybridised exclusively to 6833. Five other YACs hybridise exclusively to cosmid 5962. The YAC DNA was extracted and analysed by pulsed field gel electrophoresis followed by Southern hybridisation. This procedure confirmed that the YACs do hybridise to the markers and that they range in size from 50-240kb. Subsequently, the YAC DNA was cleaved with restriction enzymes and analysed by Southern hybridisation using the RFLP markers as probes. The pattern of hybridisation that we obtained allowed us to place the YACs in contigs. The contig which includes chalcone synthase and 6833 is approximately 300kb in length, while the one around 5962 is approximately 160kb long.

"We have identified 37 recombinants..."

Currently, we are attempting to join these two contigs. To achieve this, we are using the inverse polymerase chain reaction (IPCR) to create YAC end probes. These will be used to determine whether our present contigs already overlap. If they do not then the end probes will be used to probe the YAC library to identify YACs that span the gap between the contigs. The next step in cloning *fg* will be to locate the gene on the YAC contig. To do this we are screening for recombinant plants in which a cross-over has taken place in the vicinity of *fg*. The plants in which we are screening for recombinants have been constructed such that these recombination events will take place between chromosomes which originated in different *Arabidopsis* lines. If the YAC contig spans the cross-over breakpoint then probes from one end of it will detect RFLPs characteristic of one line of *Arabidopsis* while the other end will detect RFLPs expected from the second line. This will permit us to position our YACs relative to *fg*. So far, we have identified 37 recombinants containing cross-overs distal of *fg*. Twenty four are in the 7cM interval between *fg* and *ch7*, 13 are in the 3.2cM interval separating *fg* and *lu*. Proximal to *fg* we have isolated 4 recombinants in the 0.3cm interval between *fg* and *alb-2*. We are just beginning to analyse the DNA from these recombinants.

J. Putterill, F. Robson, K. Ingle & G. Coupland; JI Centre for Plant Science Research, Cambridge Laboratory.

*From Kevin Pyke...*

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

Since the last report we have made substantial progress in screening $M_2$ seedlings for chloroplast division mutants. We have developed a semi-automated screen using image analysis based on staining cell preparations »

with iodine. The number of chloroplasts in cells can then be automatically determined and the data for each M2 seedling compared with wild type. Any plants which have significantly more or significantly fewer chloroplasts are retained. We have screened 3000 M2 lines and found about 20 lines of interest. Of these four are so far through the $M_3$ generation. We intend to take them to $M_5$ before fully characterising the cellular phenotype and the developmental programme.

Rachel Leech (PI) & Kevin Pyke (RA); Department of Biology, University of York, Heslington, York.

*From Chris Raines...*

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

Success at last! Philip Horsnell (AFRC PMB post-doc) has constructed an *Arabidopsis* (C24) cDNA library in the vector λ zap.

"This cDNA library, plus necessary cells, is available..."

This library has been screened for three enzymes functioning in the Calvin cycle of photosynthesis. Philip has isolated full length clones encoding phosphoribulokinase and a potentially full length fructose-1,6- bisphosphatase clone. Nicola Willingham (SERC Ph.D student) has isolated a number of potentially complete clones encoding sedophetulose-1,7-bisphosphatase.

This library, plus necessary cells, is available from myself or Philip.

C.A. Raines, N.R. Baker & M. O'Farrell; Dept. of Biology, University of Essex.
RAINESC@UK.AC.AFRC.ARCB

*From Colin Robinson...*

## Isolation and analysis of *Arabidopsis* chloroplast biogenesis mutants.

During recent months, we have commenced screening of M2 seedlings for chloroplast-protein-import mutants, using a western blotting technique to detect accumulated precursor forms of light-harvesting chlorophyll-binding protein. We have also raised high-titre anti-sera against two thylakoid lumen proteins, and these will be used in conjunction with the LHCP antiserum in future screening. During this time, we have also carried out a systematic examination of the synthesis of the three proteins, in order to maximise the probability of detecting the accumulation of precursor proteins during high rates of synthesis.

Colin Robinson; Biological Sciences, University of Warwick.

*From Steve Slocombe...*

## Study of the cytochrome $b_5$ component of fatty acid desaturation

The main area of study in this project relates to the role of cytochrome $b_5$ in fatty acid desaturation in the developing embryo. An understanding of seed-specific expression of cytochrome $b_5$ in comparison to that of the leaf could enable the down-regulation of desaturation in the seed alone, by the use of seed-specific cytochrome $b_5$ anti-sense constructs. This approach, thus avoiding the potentially lethal effect of reducing desaturation in the leaf, may lead to the development of a *Brassica napus* strain that synthesizes only oleic acid in the seed. Such a desaturation-deficient crop would be of considerable commercial value. The aim was to carry out anti-sense studies initially involving *Arabidopsis* due to its lower gene copy number and ease of transformation. Attempts to derive the plant cytochrome $b_5$ gene by using a cloning approach based on animal sequence data have proven to be unsuccessful. Currently, oligonucleotide probes based on plant cytochrome $b_5$ N-terminal sequence data are being tested for screening purposes.

Steve Slocombe & Denis Murphy; John Innes Centre for Plant Science Research, Norwich.

*From Alison Smith...*

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

We are continuing with the isolation and characterisation of clones for hydroxymethylbilane synthase. We have decided to try to find a cDNA clone rather than a genomic clone initially, to make identification of open reading frames easier. In this respect, we are extremely grateful to Christine Raines for the sample of her leaf cDNA library in λ zap.

Another project which we are about to start with a new graduate student, Ashley Cook, is to try to find *Arabidopsis* mutants in the porphyrin synthesis pathway (which would almost certainly be lethal) by family analysis.

This line of research was suggested by Chris Somerville, when he came to give a very successful seminar in Cambridge in September.

Alison Smith; Dept. of Botany, University of Cambridge.
AS25@UK.AC.CAM.PHX

*From Srinivas Volety...*

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

Collaborative arrangements for the exchange of *Arabidopsis* leaf lipids were made during a meeting with John Browse (Washington State) and Chris Somerville (Michigan State) which was held at the Ninth International Symposium on Plant Lipid Biochemistry at Wye College in July. Protocols have been set up for the extraction and separation of molecular species of polar lipids using a sequence of chromatographic steps which will yield sufficient quanties of lipid for the various biophysical studies. Semisynthetic methods may have to be explored in situations where the total amount of certain molecular species of membrane polar lipids are limiting. We have also set up growth facilities at King's and expect our first harvest in the near future.

Srinivas Volety & Peter Quinn; Biomolecular Sciences, King's College London
UDBC600@UK.AC.KCL.CC.OAK

# Project Summaries

*From Mandy Walker...*

## Trichome Differentiation in Arabidopsis: Molecular Characterization of the TTG locus.

The TTG (transparent testa, glabra) locus plays an important role in a number of different pathways. Mutations at this locus result in plants which lack anthocyanins (purple pigments) as well as trichomes. The testa structure is also affected in these mutants.

We are collaborating with David Marks and Jeffrey Esh (Lincoln, Nebraska) to clone the TTG locus. Marks and Esh have already isolated a large number of recombination events between ttg and msl. We will be using these to map DNA probes from this region of the chromosome.

Another approach that we are taking is to use the anthocyanin regulatory genes of maize as probes in case there is homology between these and TTG.

The aim of this project is to understand how the gene product of the TTG locus affects trichome development from a molecular angle.

Mandy Walker & John Gray, Botany Dept., Cambridge University.
ARW13@UK.AC.CAMBRIDGE.PHOENIX

*From Felicity Watts...*

## Generalised technique for cloning *Arabidopsis* genes involved in complex biochemical pathways.

We have recently moved into newly refurbished labs., and are getting settled in. We have set up a collaboration with a plant geneticist, Dr Sandy Thomas, SPRU, University of Sussex, and will begin to analyse *Arabidopsis* plants grown from mutagenised seeds. Work is progressing on our search for cell cycle and mitochondrial genes.

Felicity Watts, Tony Moore & Julian Burke; University of Sussex.

*From Zoe Wilson...*

## Genetic male sterility in *Arabidopsis*.

Since the last report Zoe Wilson and Mark Aarts (an ERASMUS student from Wageningen) have been setting up the chromosome walking methodology following the arrival of the Somerville YAC library and many tips gained by a visit to Norwich (thanks Caroline, Gerda, Renate, Ian *et al*). RFLP probes have been donated by the Goodman and Meyerowitz labs so we are now starting a walk around msl. Despite kanamycin selection (50 mg/ml), mini-preps from these RFLP clones have revealed high levels of internal recombination, making the screening of large numbers of colonies essential. (This agrees with Renate *et al*'s findings).

> "...develop a seed mutagenesis protocol based on gamma-irradiation."

As part of this process, for the last few months we have been screening for recombinants around msl (in the progeny of a cross between parents coco ch7ch7 (Columbia) and ttgttg msl msl (Landsberg *erecta*)). These recombinants should prove invaluable in fine mapping the region of chromosome 5 around msl and in working out which way the chromosome walk is going. Fortunately, as Newsletter readers will know, there is a lot of moral and practical support in the AFRC Programme for those interested in walking in the top arm of chromosome five!

We have been following the CTAB DNA isolation procedure modified by Ian (as enclosed in the last Newsletter) and have found it works well, but for our lines the addition of 1mM spermidine was essential for successful restriction digests.

Because our first small scale seed mutagenesis experiment was done (yes, Bern actually did something in the lab.) with X-rays we were very interested to read about recent developments in genomic subtraction. Mark Aarts has been using a new 137Cs source in the Botany Department to develop a seed mutagenesis protocol based on γ-irradiation. We hope to say more at the Xmas meeting about progress.

Zoe Wilson, Janet Fuller, Jane Russell, Mark Aarts, Greg Briarty & Bern Mulligan; Dept. of Botany, Nottingham University. MAC and MODEM available. Tel. 0602-484848 (ext 3467).
PBXZW@UK.AC.NOTTINGHAM.CCC.VAX

## Guest Summary

*From Jo Burn...*

I have just taken up a postdoctoral position in Liz Dennis's group at CSIRO, Canberra to investigate the molecular basis of the vernalization requirement of certain ecotypes of *Arabidopsis*. The work done so far has been carried out in collaboration with David Bagnall from the Physiology group at CSIRO. David has determined the vernalization response of a number of ecotypes from the Brock/Langridge *Arabidopsis* collection. To date, three late flowering ecotypes have been identified, in which the time to flowering is considerably reduced in response to a cold treatment of about 30 days. These have also been fully characterised with respect to their response to differing light quality and intensity, and to varying sugar concentrations. Now that the growth conditions for these ecotypes have been determined, the genetics of the vernalization response will be investigated by carrying out crosses between each of these three ecotypes, and early flowering *Arabidopsis* ecotypes, such as Landsberg and Columbia. A second approach that is planned, is to screen for late flowering mutants (following an EMS mutagenesis of Landsberg *erecta* seed) under balanced/ incandescent light. This should (hopefully!) count against picking up already characterised late flowering mutations, such as fca, which appear to be sensitive to light quality.

Joanne Burn, David Bagnall & Liz Dennis; CSIRO, Division of Plant Industry, GPO Box 1600, Canberra, ACT 2601, Australia.
BURN@AU.OZ.PICAN

**Please send your next summary to the newsletter by Monday, 4th February, 1991 at the very latest.**

# Trivial Pursuits

## Stanzas for Scientists

You could be forgiven for thinking that this issue's poetry was to be found in Andy Maule's project summary. However, thanks to Keith Roberts from John Innes we have some more poetry with a scientific basis. To quote Keith: "I appreciated the "Stanzas for Scientists" section and saddened at the zilch response, so I enclose two poems by Miroslav Holub (born in 1923). He is one of the leading Czech poets, but professionally is a distinguished research scientist, originally a clinical pathologist and lately, I believe, in the immunology area. He does much, in a long series of poetry volumes, to deny the apparent hegemony of C.P. Snow's "Two Cultures" position." *(Lord Snow's views can perhaps be summarised as, scientists don't read and humanists don't know what entropy means – ACM.)*

For trivia buffs, Keith also writes, "Did you know that *Thalia* was the muse of pastoral and comic poetry? She was also one of the three graces, a patroness over festive meetings." A good omen for the Nottingham meeting?

*Wings*

We have
a map of the universe
for microbes,
we have
a map of a microbe
for the universe.

We have
a Grand Master of chess
made of electronic circuits.

But above all
we have
the ability
to sort peas,
to cup water in our hands,
to seek
the right screw
under the sofa
for hours

This
gives us wings.

*In the Microscope*

Here too are dreaming landscapes,
lunar, derelict.
Here too are the masses
tillers of the soil.
And cells, fighters
who lay down their lives
for a song.

Here too are cemeteries,
fame and snow.
And I hear murmuring,
the revolt of immense estates.

## T-shirt Competition

Entries have been trickling in for the T-shirt competition. Thank you to all those making the effort to create and send in designs. Standards of pen-work and originality have been high and, in general, not too libellous. If all goes to plan, the winning logo will be selected and printed in time for the Christmas meeting. The judges meet in a couple of weeks and so there's still time for last-minute entries. As an incentive, the prize is a free T-shirt and a bottle of something sparkling (hopefully containing alcohol and not benzene).

## This Issue's Quote

Renate Schmidt was the first person to recognise the author of the quotes featured in the last issue: a "Weissbier" is now hers for the asking. If anyone out there didn't work out who it was, then send a plain brown s.a.e to the usual newsletter address and all will be revealed. Also send your suggestions for quotes to that address. This issue's quotes were both sent in by Keith Roberts from John Innes. The first is highly appropriate to Arabidian research and is from, *Fortune of the Republic*, by Ralph Waldo Emerson:

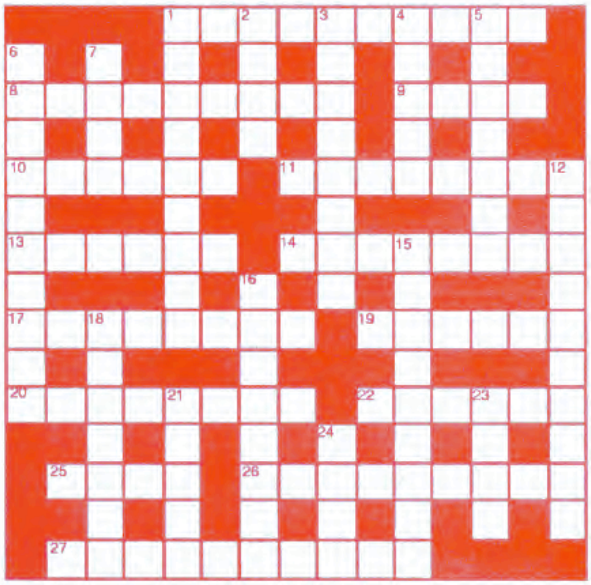
"What is a weed? A plant whose virtues have not been discovered."

Just mention where you got it from the next time you use it in a talk.

The second quote is probably much more likely to be appreciated by non-Arabidian researchers. The ACM is sure that you all recognise the words of Shakespeare's *Othello* (Act IV, Scene II):

"O thou weed,
Who art so lovely fair and smell'st so sweet
That the sense aches at thee, would thou hadst ne'er been born!"

## *Arabidopsis* Prize Crossword



## 'ere we go!

by Black Rot

FOR THOSE INTERESTED, Dick Flavell (see the photo on page two) is picking out the winner of the last newsletter's prize crossword. The lucky entrant was none other than **Alison Smith**, from Cambridge University. As she is a grant holder, this leaves a certain amount of egg on the ACM's face (sadists please refer to the bottom of page 14 in *Th'ale & Cress*). On the other hand, at least the response to the second crossword was much less underwhelming than last time. Five correct answers were received -- a 500% increase in real terms, even allowing for seasonal adjustments. The incentive of a site prize of a bottle of wine probably aided this figure by flushing out two entrants from the three John Innes Centre labs. (Mary Knights, from the Cambridge Laboratory, was the winner of this sub-competition and is now the proud owner of a bottle of AFRC Liebfraumilch. Interestingly, she is the second "non-scientist" from the Cambridge lab. to win a prize in this hallowed competition.)

A £5 book token will soon wend its way to our latest winner. Congratulations to Alison and also to the runners up: Sue Albini (Birmingham), Barrie Allen (Cambridge lab, J.I.) and Helen Ougham (WPBS).

Spurred on by the increased interest in the crossword, the ACM has again persuaded Black Rot to produce yet another gem. The tip this time is that clues 1, 8, 20, and 27 across and 3, 4, 5, 6, and 12 down are all linked in a common theme, which is expressed in the title: *'ere we go!* Our fiendish setter also warns us that the answer to 19 across is somewhat obtuse. (Although the ACM got it on his test run, but struggled with 16 down. This may be more revealing about the ACM, however, than the crossword.) Another book-token for the first correct entry.

### Clues Across

1. City's rest disturbed by French channel proposal (10)
8. Homogenised plover oil provides champion qualities (9)
9. Warning given about Verne's captain (4)
10. Persian notable returns old king to backward female (6)
11. U -- accede to rearrangement about physical jerks (8)
13. Eamonn's confused by Clint's rôle (2,4)
14. Point to coach for coarse filter (8)
17. Americanism has Madrid's team on Riviera (4,4)
19. Oranges and lemons for Pavarotti ? (5)
20. Wigan (or Oldham) showing sporty quality (8)
22. Insect contributes to clear Wigan's pier? (6)
25. Employed American journalist (4)
26. Do abstain perhaps from punishment (9)
27. Stanley in old team of Lancastrians (10)

### Clues Down

1. Centre forward as assassin's target ? (6,3)
2. Light element ? (4)
3. East coast boat people ? (4,4)
4. Fuel the furnace in the Potteries (5)
5. Toffees are green in age ? (7)
6. Girl one found in Crewe (10)
7. Always part of 5 down (4)
12. Grace is overweight in the NE (10)
15. Hair style on Indian from Uganda ? (4-5)
16. Brian endlessly sick about composer (8)
18. As out of step as chap I confused (7)
21. Duck down (5)
23. NE flower has obstructions you say (4)
24. Aint proper (4)

---

IF YOU ARE STILL grinding your teeth at the two clues you couldn't get last time, here are the answers to *Weiner Schnitzel*, the crossword in Tha'le & Cress:

Rules of the *Arabidopsis* Prize Crossword:
This competition is open to all readers of this newsletter. In the ever-increasing likelihood of a grant holder supplying the first correct entry, a project summary must have been received for them to be eligible for the grand prize. The answers as well as the winner's name will be in the next newsletter.