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TRANSCRIPTIONAL ANALYSIS OF THE R LOCUS

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Progress Report

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TRANSCRIPTIONAL ANALYSIS OF THE R LOCUS OF MAIZE

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The R locus contols where, when and how much anthocyanins are expressed in at least 11 different tissues of the corn plant and seed. Enormous natural variation has been seen when the phenotypes of different R alleles are compared in a common genetic background. Some alleles have been shown to have a compound structure resulting from gene duplication and divergence. In these complex alleles, each member of the duplication (called R genic elements) has a unique pattern of expression. The function of the R locus is not known; genetic and biochemical analyses suggest that it may encode a protein that regulates other genes in the anthocyanin pathway.

Over the past year we have determined that the genic elements (P), (S), and (Lc) all encode a very rare 2.8 kb transcript that is present in tissue displaying anthocyanin pigmentation. cDNA libraries have been constructed using mRNA isolated from tissues shown by Northern blots to be enriched for the R transcript. Full-length cDNAs will be sequenced and compared to each other and to the genomic sequences being determined at Yale (Dellarpota). In addition, these cDNAs will be a starting point in the eventual isolation of antibody that will be used with the cDNAs to localize R gene products and their kinetics of accumulation. This will then be correlated with the presence or absence of pigment.

Progress Report for Grant # DE-FG09-86ER13621

Transcriptional Analysis of the R Locus

Susan R. Wessler, Principal Investator

Time period covered in this report:

Even though this report covers the 18 month period beginning September 1, 1986, the work described was actually initiated in March, 1987 when Dr. Steve Ludwig, a postdoctoral associate, arrived in the lab. So, this report actually covers the 8 month period between March and October.

Previous attempts to analyze R transcription and isolate cDNA clones:

Northern blot analysis of coleoptile tissue isolated from the R-r allele (obtained from Jerry Kermicle, as are all of our strains) revealed a 2.8 kb transcript when 20ug of polyA+ RNA was probed with a labelled 700 bp R-nj probe (provided to us by Steve Dellaporta, as are all of our genomic DNA probes). This RNA was used by Dr. George Baran (an NIH postdoctoral fellow) to generate a cDNA library which was probed with labelled R-nj DNA. Six positives were obtained and 4 appeared to be full length (they had inserts of about 2.8 kb).

These 6 positives were analyzed further when Dr. Ludwig arrived in the lab. He generated unidirectional deletions of the large clone, sequenced part of the insert and, we were embarrased to find, the clone was an artifact - it was similar to pBR sequences. One of the 5 remaining cDNA clones was shown to be substantially shorter (about 700 bp) and hybridized specifically with the R-nj insert. Again, unidirectional deletions were made and these templates were sequenced. To ensure that this too was not an artifact, we sequenced the entire R-nj genomic insert (about 750 bp) and were distressed to find that the sequence of the cDNA and the genomic was identical. Apparently, in one of the more bizarre artifacts I have seen, the cDNA primed on a poly A stretch in the R-nj insert which, for some reason, was contaminating our mRNA preparation.

In any event, we have the sequence of a short region of R-nj genomic DNA. Computer translation of this sequence reveals an extended open reading frame which may not be very significant since this is genomic DNA.

Current (and more careful) Progress

Dr. Ludwig was, needless to say, a little upset about wasting his

time sequencing someone elses artifacts. His last 6 months have seen considerable progress that can be summarized as follows: (1) He has isolated numerous tissues from a variety of R alleles, looking first for an mRNA that is unambiguously an R transcript and after that, an abundant source of this transcript.

The (S) genic element: A 2.8 kb transcript has been detected in aleurone tissue isolated 32 days after pollination (DAP) from a strain harboring (S) but not (P). No transcript was detected in the aleurones of tissue isolated when the color first appears with this allele (about 14 DAP) or when there is substantial aleurone color (at 18 DAP).

The (P) genic element: A 2.8 kb transcript has been detected in the red anthers (2 days prior to pollen release) of a strain harboring (P) but not (S).

The Lc genic element: A 2.8 kb transcript has been detected in the glumes of unfertilized female flowers. This transcript was detected in a strain designated R-g:1 Lc, this strain was also recessive for pl, so plant color was light dependent. The 2.8 kb transcript was detected whether the ear was exposed to light (red glumes) or not (colorless). So, the presence of the transcript is not necessarily correlated with the presence of anthocyanin and the R transcript is not induced by light. The 2.8 kb transcript is also detected in the red pericarps of these strains.

Although we have not as yet quantified this transcript, it is quite rare except in the female glumes of strains harboring Lc. In fact, we were not able to detect the (P) or (S) transcripts using nick translated probes labelled to 5 X 10^8 cpm/ug. To detect these, 5ug of polyA+ RNA was probed with strand specific probes using the method of Hu and Messing (1982, Gene 17: 271-277). These strand specific probes also were used to demonstrate that this transcript only hybridized with one strand and that this was the one complementary with the large open reading frame described above. Furthermore, in addition to using the R-nj probe, the above transcripts have been shown to be homologous with (P) and (S) genomic subclones provided to us by Steve Dellaporta.

The R-nj allele: In order to complete our manuscript on the cloning of the R-nj allele, I have been trying to detect a transcript encoded by this allele. To this end I have assayed embryo and aleurone tissues at 15, 25, and 35 DAP in addition to coleoptile and red roots tissues. I have not been able to detect a R-nj transcript. The last tissue remaining to be tested are the anthers where there is substantial anthocyanin in this allele.

A correlation between the amount of anthocyanin and the 2.8 kb transcript

We were surprised that the R transcript could only be detected in 32 DAP aleurones and not in 18 DAP tissue, which appears visually to be just as darkly pigmented. However, a review of the literature (especially Styles et al., 1973 Can. J. Genet Cytol. 15: 59-72) indicates that aleurone pigment continues to accumulate until 45 DAP. In fact, at 45 DAP there is almost 100 times more anthocyanin than at 20 DAP. Our very preliminary analysis indicates a similar accumulation for the R transcript.

The accumulation kinetics of anthocyanin pigment in the aleurone may also explain my failure to detect a R-nj transcript in this tissue. According to Styles et al., there is at least 10 fold less aleurone pigment in R-nj than in R-r. Considering the weak Northern signal we see with R-r material, the R-nj at 10 fold less would not be detectable.

Construction of cDNA libraries

Dr. Ludwig has just constructed 2 cDNA libraries utilizing polyA+ RNA from female glumes (Lc), and anthers (P). These libraries are just now being screened for plaques homologous to the Lc and P genomic probes.

Analysis of other R alleles

We have obtained over 100 different R alleles from Jerry Kermicle. The tissue specific pattern of expression of these alleles was characterized by Brinks student W. van der Walt. What is nice about this collection is that it represents a diverse set of alleles and, more importantly, all have been backcrossed into a common genetic background for at least 5 generations. We have revived these strains from 10 to 25 years in cold storage and, over the next year, we will begin to focus on the more extreme phenotypes for further analysis.

We have also obtained from Jerry Kermicle a collection of interesting R alleles studied by Seymour Fogel when he was a student with Louis Stadler. The tissue specific pattern of expression of these alleles has been carefully characterized in Fogel's Ph.D. thesis. These alleles will also be evaluated for further analysis.

Finally, Steve has analyzed different R alleles when they are combined with certain mutant alleles which transform one tissue type into another. For example, some of the knotted alleles result in the formation of displaced ligules. By combining Lc (which conditions anthocyanin formation in the ligule) with a particular knotted allele, he was able to see very red displaced

ligules. This shows that it is something about ligule tissue that turns R on rather than where the ligule is on the plant.