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FINAL REPORT

The classical maize mutant *iojap* (*Iodent japonica*) has variegated green and white leaves (Jenkins, 1924). Green sectors have cells with normal chloroplasts whereas white sectors have cells where plastids fail to differentiate. These mutant plastids, when transmitted through the female gametophyte, do not recover in the presence of wild type *Io* (Rhoades, 1943). We cloned the *Ij* locus, and we have investigated the mechanism of epigenetic inheritance and phenotypic expression (Han et al., 1992). More recently, a modifier of this type of variegation, *Inhibitor of striate*, has also been cloned (Park et al., 2000). Both the *iojap* and *inhibitor of striate* proteins have homologs in bacteria and are members of ancient conserved families found in multiple species. These tools can be used to address fundamental questions of inheritance and variegation associated with this classical conundrum of maize genetics.

Since the work of Rhoades there has been considerable speculation concerning the nature of the *Io* gene product, the origin of leaf variegation and the mechanism behind the maternal inheritance of defective plastids. This has made *iojap* a textbook paradigm for cytoplasmic inheritance and nuclear-organelle interaction for almost 50 years (eg. Goodenough, 1978). Cloning of the *Io* gene in maize, and homologs in other plants and bacteria, provides a new means to address the origin of heteroplastidity, variegation and cytoplasmic inheritance in higher plants.

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SUMMARY OF PROGRESS.

We have determined the structure of a number of mutant alleles at the *Ij* locus in maize, including *ij-mum17* which caused by the insertion of a *Mutator* element in the upstream region. This allele is suppressible so that a mutant phenotype is only observed in homozygous *ij-mum17* plants when *Mu* elements are active and unmethylated. We have identified and mapped a homolog of the maize *Ij* gene in *Arabidopsis*, and we have

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identified a bacterial homolog of *Ij* in a subset of eubacterial genomes, including the cyanobacterium *Synechocystis*, and *E.coli*. We have constructed a deletion in *E.coli*, and we have identified a transposon 700bp upstream of *Ij* in *Arabidopsis*. We have purified 6 different recombinant *Ij* fusion proteins, and begun to characterize their properties. We have raised polyclonal and monoclonal antibodies to the *Arabidopsis* and maize proteins. The *Ij* protein in maize is plastid localized and associated with large protein complexes including 50S plastid ribosomes. We have constructed genetic strains to determine the developmental basis for maternal inheritance and the pattern of variegation. We have examined the interaction with second-site modifiers of variegation in maize, such as *Inhibitor of striate*. This gene has been cloned by tagging and also found to be conserved in bacterial genomes.

1. The *Ij* locus in maize and *Arabidopsis*.

A map of the *Ij* locus in the inbred B73 (Han et al., 1992) is shown in Fig.1A. Transcript mapping using subcloned fragments revealed the presence of 2 genes: the *Ij* gene comprises 5 exons encoding a 26kD precursor protein. Approximately 3 kb upstream, a second gene was discovered by transcript mapping (named *Fgh*, after "Fgh..*ij*"). *Fgh* has a single exon which encodes a 740 aa protein with homology to the *no apical meristem* family of putative transcription factors (Souer et al., 1996; Aida et al., 1997). Southern blotting and library screening, even at reduced stringency, failed to detect additional *Ij*-like genes in maize. This portion of chromosome 7L may not have been segmentally duplicated, as the genes *glossy1* (Hansen et al., 1997), *iojap* (Han et al., 1992) and *ramosa1* (our unpublished results) are not duplicated at this level of resolution. This is in contrast, for example, to the *hcf106* locus on chromosome 2S, which is functionally duplicated on chromosome 10L (Settles et al., 2000).

The *ij-mum1* and *ij-ref* alleles are as described in Han et al. (1992). *ij-mum17* arose by transposon mutagenesis (Byrne and Taylor, 1996), and has an insertion located upstream of the *ij* transcription unit. *Mu* insertions in the 5' regulatory region often result in a suppressible mutant phenotype (Martienssen, 1996). That is, the mutant phenotype is only apparent in homozygous seedlings when *Mu* elements are active and unmethylated. *ij-mum17* plants were selfed and normal green plants were identified among their progeny (Fig. 3A). These phenotypically suppressed plants had lost *Mutator* activity and gave rise to normal progeny when self-pollinated, but mutant progeny when backcrossed to homozygous plants that had retained *Mu* activity. This standard test (Martienssen et al., 1990) confirmed that *ij-mum17* was suppressible. We can use this feature of the *ij-mum17* allele to perform mosaic analysis of *ij* function (Martienssen and Baron, 1994) as described below.

The *Arabidopsis Ij* homolog in *Arabidopsis thaliana* (*AtIj*) was identified by screening cDNA libraries using the maize *Ij* gene as a probe (Byrne and Taylor, unpublished). The predicted protein sequence is 64% identical with the maize sequence (Figure 2), except for the first exon, which is thought to encode a transit sequence that is cleaved during chloroplast import (Han and Martienssen, 1995). The first exon in *Arabidopsis* encodes 82 aminoacids with a serine-rich composition consistent with this role. The *Arabidopsis* cDNA was mapped to chromosome 3, approx. 10 map units north of chloroplast mutator (*chm*), using YAC libraries and recombinant inbred lines. The *AtIj* gene is single copy. The *Arabidopsis* genome sequencing project has recently confirmed these results, placing *AtIj* on chromosome 3 P1 clone MGH6. There is no sign of an *Fgh*-like gene on either side of the *Ij* locus in *Arabidopsis*. The full length *Arabidopsis Ij* cDNA sequence was used to generate antisense plants (M. Byrne and W.C. Taylor, unpublished). Three plants were found with a variegated phenotype, indicating that *AtIj* is likely to encode a functional homolog of the maize gene (Fig 3B and C). The phenotypic variation between the three transgenic lines

may reflect different degrees of antisense suppression, and heritability was difficult to demonstrate.

2. Overexpression, protein purification and antibodies.

We have generated a number of fusion proteins with both maize and *Arabidopsis* Ij (Fig. 4). Antibodies were raised against the GST-Ij fusion, which encodes the N-terminal 60% of the mature Ij protein, and purified using the TrpE-Ij fusion protein. Quantities of this purified protein were limited so antibodies have also been raised against the His-Ij and His-AtIj fusion proteins, both of which encode predicted full length mature Ij from maize and *Arabidopsis*, respectively. Antibodies to the AtIj have been affinity purified using the His-AtIj fusion protein. This antibody will be used in analysis of transgenic antisense plants. Antibodies to the maize Ij are in limited quantity, but can be used for most purposes including immunolocalization (Han and Martienssen, 1996). We have raised a panel of 15 monoclonal antibodies against the maize Ij fusion protein, each of which has been shown to detect maize Ij protein *in vivo*. Interestingly, a subset of these antibodies detect a second protein, about twice as large as Ij, that is only found in wild-type and not in homozygous mutant leaf tissue.

The *Arabidopsis* His-AtIj fusion protein is abundantly expressed in *E. coli*, but sequestered within inclusion bodies. We obtained soluble, highly purified protein by solubilization with urea, affinity purification in Ni-NTA resin and subsequent removal of the urea by dialysis. Fractionation through an ion exchange column yields a highly purified fusion protein. Analysis of the fusion protein with a molecular sizing instrument, operating on dynamic light scattering, showed a single peak with a radius of 8.7nm. The large particle size is calculated to have a molecular weight of between 550 and 570 kDa. The presence of only a single molecular species is a strong indication that this large molecular weight particle is not a result of non-specific aggregation (Leemor Joshua-Tor, personal communication). We will confirm these results by first clipping off the His tag at the protease cleavage site provided in the His vector system, as the tag is known to cause artificial association in some instances. Nonetheless, our preliminary results raise the possibility that Ij self-associates *in vitro*. However, sucrose gradient fractionation suggests that the recombinant fusion protein fractionates differently from native Ij in leaf extracts and is associated with other proteins *in vivo*.

3. Association with other proteins in wild-type and defective plastids

We have shown that Ij is localized in the chloroplast, and is associated with the 50S subunit of the chloroplast ribosome in leaf and chloroplast extracts run on sucrose gradients (Han and Martienssen, 1995). This association is at least partially sensitive to puromycin and EDTA and resistant to high ammonium salt concentrations. To further test this proposal we have tested for the association of Ij with proteins in the mutant *crs2* (*chloroplast RNA splicing 2*) and in exceptional *ij* maternal white seedlings. *crs2* shows a global defect in chloroplast splicing and lack of *rps16* and *rpl16* mRNA. This results in chloroplasts without ribosome function (Jenkins et al., 1997). Maternal white seedlings that have inherited white, *ij* affected plastids from their maternal parents also lack ribosomes (Walbot et al., 1979). The *crs2/crs2* and *ij/+* maternal white seedlings are much reduced in Ij protein compared to wild-type plants, but in both mutants the remaining Ij protein does not sediment in a sucrose gradient (Fig. 5). This observation is consistent with absence of chloroplast ribosomes in these seedlings.

4. Ij homologs in bacterial genomes

Chloroplasts evolved from endosymbiotic photosynthetic bacteria engulfed by primitive protists. For this reason, many genes involved in fundamental aspects of gene expression, replication, division and chloroplast protein trafficking have been conserved. For example, the *hcf106* gene of maize encodes a chloroplast membrane protein required for protein translocation (Settles et al., 1997). Homologs of *hcf106* have been identified in each bacterial genome (the *tat* genes), and disruption in two different

species results in secretion defects in bacteria consistent with the role of Hcf106 in chloroplast membrane assembly (Settles and Martienssen, 1998). Chloroplast ribosomes are closely related to those of bacteria and only three out of 44 chloroplast ribosomal proteins have been found with no reported sequence similarity to any bacterial ribosomal protein (Harris et al., 1994). We have found that one of these three, S30/S22 does in fact show significant similarity to a bacterial protein that modulates the sigma 54 transcriptional activator (Tan et al., 1996).

In database searches using BLAST (Altschul et al., 1990) we have found a number of bacterial *Ij* homologs. These hypothetical proteins share between 23-37% identity and 50-57% similarity with the predicted mature maize *Ij* (Blast P(N) scores 3.2×10^{-20} to 1.2×10^{-2}). A small number of residues are identical for all of these small proteins (Figure 2). These could define amino acids critical for function of *Ij*. Mycobacteria and archaeobacteria do not have *Ij* homologs (Fraser et al., 1995; Bult et al., 1996) perhaps because the translation apparatus differs from the eubacterial paradigm. Hypothetical proteins in *C. elegans* and *Drosophila* share very weak similarity with maize *Ij* but have all of the seven amino acid residues conserved in the bacterial sequences (Figure 2). The *C. elegans* protein may be mitochondrial as an N-terminal extension is suggestive of a mitochondrial transit peptide. However, no *Ij* homologue appears in the genome of the yeast *S. cerevisiae*, suggesting that yeast mitochondria at least can function without *Ij*.

More recently, Tatusov et al. (2000) have defined ancient conserved domains common to subsets of the 22 complete bacterial genome complements so far determined. *Iojap* is found in 14 of the genomes (http://www.ncbi.nlm.nih.gov/cgi-bin/COG/palog?BS_yqeL), and cluster analysis reveals that the subset of organisms that share *Ij* also share a limited number of protein classes in common (Table 1). The significance of these clusters is discussed below.

The *E. coli* gene *ybeB*, which shares similarity with maize *Ij*, is the first gene of a translationally coupled operon consisting of *ybeB*, *ybeA*, *pbpA* and *rodA* (Asoh et al., 1986; Matsuzawa et al., 1989). *pbpA* and *rodA* encode genes required for proteoglycan cell wall synthesis and cell shape and disruption of the *pbpA-rodA* region is lethal in wild type *E. coli* cells. However, mutations in aminoacyl-tRNA synthetases suppress this lethality by inducing the stringent response, so providing a link between growth rate, ribosome activity, and cell wall synthesis (Vinella et al., 1993). The *E. coli* stringent response results in pleiotropic effects on RNA accumulation and translational fidelity, and is induced under a variety of nutritional and physiological stress conditions. It is largely dependent on the activity of a ribosome-associated ppGpp synthetase (*relA*) that generates the nucleotide signaling molecule that mediates the effect (reviewed in Cashel et al., 1996). Recently, Arabidopsis homologs of *relA* were identified in a two-hybrid screen. Although these proteins have S-rich N-terminal extensions that resemble plastid import signals, the possibility that they were plastid localized was not considered because they interact with the disease resistance receptor RPP5 (van de Biezen et al., 2000).

The gene order of the potential *rodA* operon is conserved in *H. influenza*, but unrelated genes occur downstream of the *Ij* homologue in all other bacterial genomes including cyanobacteria (Fig. 1B). Nonetheless, it is tempting to speculate that the selective lethality of *pbpA* in *E. coli* may be related to a conditional requirement for *Ij*. Further, some aspects of the stringent response display epigenetic inheritance in that induced cells display lasting physiological changes, such as antibiotic resistance (Boulloc et al., 1992).

In addition to *ybeB*, the *nusG* gene of *E. coli* is also related to the C terminal domain of *Ij* (Fig 2). By way of comparison, the *E. coli* gene *rfaH*, and the yeast gene *spt5*, have both been identified as homologs of *nusG* on the basis of much weaker amino-acid similarity, and have biochemical roles consistent with the homology (Bailey et al., 1996; Hartzog et al., 1998). *NusG* is essential in *E. coli*, but dispensable in *B. subtilis* (Ingham et al., 1999). It is required to efficiently terminate transcription of ribosomal RNA, and mutants fail to accumulate the mature

transcript. Mature rRNA is severely depleted in *iojap* plastids (Walbot et al., 1979; Han et al., 1993), raising the possibility that *iojap* may have a similar role in chloroplasts (Sriraman et al., 1998).

Polyribonucleotide nucleotidyltransferase
 Predicted pseudouridylate synthase family 1
 Protein chain release factor B
 Ribosomal protein L25 (general stress protein Ctc)
 Transcription termination factor NusB
 Primosomal protein N' (replication factor Y) - superfamily II helicase
 Bacterial cell division membrane protein FtsW/MrdB/SpoVE
 Protease subunits of ATP-dependent proteases, ClpP family
 Cell division protein FtsI/penicillin-binding protein 2 pbpA
 D-alanine-D-alanine ligase and related ATP-grasp enzymes
 UDP-N-acetylglucosamine enolpyruvyl transferase
 UDP-N-acetylmuramate dehydrogenase
 UDP-N-acetylmuramate-alanine ligase
 UDP-glucuronosyltransferases
 Co-protoporphyrinogen III oxidase and related FeS oxidoreductases
 Predicted nucleotide-binding protein, YjeE family
 Uncharacterized CBS domain-containing proteins
 Uncharacterized ACR (homolog of plant *iojap* proteins)

Table 1. Genes unique to *iojap*-containing microbes.

Remarkably, cluster analysis (Table 1) reveals that peptidoglycan biosynthetic enzymes (including pbp2), the nusG interacting protein nusB, and ribosome release factors are all unique to the subset of microbes that contain IJ (Table 1). This suggests that IJ is involved in some aspect of gene regulation common to this set of responses.

5. Plastid gene expression in maternally defective plastids.

We have investigated the pattern of chloroplast gene expression in white seedlings that have inherited defective plastids from *ij/ij* homozygous plants, and compared these patterns to those found in other albino mutants in maize (Han et al., 1995). Albino mutants can be divided into class I mutants, which have white endosperm and predominantly effect carotenoid biosynthesis, and class II, which do not effect carotenoid biosynthesis *per se*, but fail to accumulate pigments in the leaf. Qualitatively, Class I albino mutants have essentially wild-type patterns of chloroplast gene transcripts (Han et al., 1995). Class II mutants, including *ij*, have highly aberrant patterns and can be divided into 2 subsets based on Northern blotting (Han et al., 1995).

We have supplied *ij* maternal defective material to A. Barkan and P. Maliga who have performed in depth studies of RNA splicing and promoter utilization, respectively, in maternally defective plastids. They have found that *ij* and some other class II mutants fail to splice a subset of chloroplast transcripts (Jenkins et al., 1997). Other class II mutants have a reciprocal pattern of splicing, which could explain the differences in accumulated transcripts observed by Han et al. (1995). Transcript mapping by primer extension has revealed that *ij* affected plastids have an altered specificity of transcriptional initiation (Silhavy and Maliga, 1998). Chloroplast gene transcription is controlled by a nuclear encoded RNA polymerase (NEP), and a plastid encoded RNA polymerase (PEP). Plastid genes are equipped with an NEP promoter, a PEP promoter, or both (Hajdukiewicz et al., 1997; Stern et al., 1997). In common with other ribosome-deficient plastids in barley, and with tobacco mutants that are deficient in the PEP itself,

transcripts in maternally defective *ij* plastids are initiated only at NEP promoters (Allison et al., 1996; Silhavy and Maliga, 1998). It is tempting to speculate that differences in splicing may reflect alternate nuclear and plastid encoded splicing factors in a similar way. However, it is also possible that splicing is the primary defect in some of these mutants, leading to loss of ribosomal proteins whose mRNAs are spliced (Jenkins et al., 1997).

These observations are consistent with the idea that chloroplast gene expression is affected indirectly in *iojap* mutants because of the loss of plastid ribosomes. That is, the inability to translate plastid encoded polymerase and splicing factors leads to alterations in plastid transcript initiation and splicing. A similar explanation has been proposed to explain splicing defects and ribosome loss in the *albostrians* variegated mutant of barley (Hess et al., 1994). Mitochondrial gene expression is also affected in barley seedlings that inherit defective plastids (Hentke et al., 1999).

6. Genetic analysis of *ij* function in maize.

We are exploring a number of genetic avenues to determining the origin of the pattern of variegation and heteroplastidity found in homozygous *iojap* plants, and we have begun to explore the mechanism by which defective plastids are maternally inherited. We have also determined the potential role of known modifiers of variegation in *iojap* striping.

Mosaic analysis in variegated leaves. Immunolocalization in thin sections reveals that the *Ij* protein is highly expressed in most cell types in immature leaves, and in the subepidermal photosynthetic tissues in the outer (older) leaves (Han and Martienssen, 1996). In order to examine the site of *Ij* gene action in the leaf, marked mosaics comprising adjacent mutant and wild-type tissue need to be constructed. Plants homozygous for the reference allele *ij-ref* are genetically mosaic because the *Ds* element interrupting *ij* excises during development leading to large and small sectors of wild-type tissue (Han et al., 1992). Revertant sectors in *ij-ref* plants appear fuzzy, or diffuse in the transverse dimension (Fig. 6). These results support the idea that the "grainy" mutant phenotype can be rescued to some extent by neighbouring wild-type cells immediately above or below a mutant cell (Rhoades, 1946; Langdale et al., 1989).

Lineage analysis. According to the "pseudochimaera" model (Coe et al., 1988) the white stripes found in *iojap* homozygous mutant plants are the result of periclinal divisions from the LI lineage that "invade" the underlying sub-epidermal layer during leaf initiation (see Background). In order to test this model, we constructed plants that were heterozygous for two genes required for anthocyanin biosynthesis: Booster (*B*) and Purple (*Pl*), and homozygous *ij/ij*. Following irradiation, clones of cells were marked in these plants by loss of anthocyanin pigmentation due to loss of the *B* (2S) or *Pl* (6L) chromosome arm (McDaniel and Poethig, 1988). By analyzing more than 200 sectors in mutant (*ij/ij*) plants and their normal heterozygous siblings (*ij/+*) we found that *b/-* sectors that occur in only one layer of the leaf (either epidermal or subepidermal) had unaffected chlorophyll pigmentation in both mutant and normal sibs. Furthermore, sectors in normal plants (*ij/+*) that were in both layers were also green. However, 89 out of 94 sectors that were in both layers in mutant (*ij/ij*) plants, were white or mottled in appearance relative to neighbouring tissues.

Sectors in both epidermal and subepidermal layers must be derived from a single cell in either the LI or the LII layer of the meristem that subsequently divided periclinally to occupy the other layer (McDaniel and Poethig, 1988). In 34 of the 89 white sectors we examined, we could demonstrate that invasion had occurred from the LI into the LII by

examination of the sector in several leaves: an epidermal sector in a lower internode frequently entered the sub-epidermal layer in an upper (and so later) leaf. In 2 out of the 5 "green" sectors, it was possible to show by similar means that invasion had occurred in the opposite direction (LII to LI). In the remaining 58 LILII sectors, the layer of origin could not be determined. These results suggest that periclinal divisions from the LI layer of the meristem into the underlying tissue result in white stripes in homozygous *ij/ij* plants. In most cases, invasions occurred in the apical meristem itself shortly after irradiation, resulting in clonal white sectors that passed from leaf to leaf. Such sectors are not apparent in non-irradiated *ij/ij* plants. It is possible, therefore, that these invasions were induced by irradiation, as has been observed in numerous cases using similar doses of X- and gamma-radiation (Tilney-Bassett, 1986; Dawe and Freeling, 1990).

We have generated additional marked sectors using the suppressible *ij-mum17* allele. Double mutants carrying the suppressible anthocyanin pigment biosynthesis gene, *a1-mum2*, were constructed. Rare plants were mosaic for both genes, and occasional sectors were observed (Figure 6). When anthocyanin pigmentation was lost in the L1 layer, resulting in a pale purple sector, the sector was also phenotypically *ij*. White stripes within these sectors were completely free of red pigmentation, suggesting that they penetrated to the underlying layer, as predicted by the pseudochimera model (Fig. 6). We will test many more plants of this genotype to confirm this model.

Investigation of the maternal inheritance of defective plastids. Homozygous mutant *ij/ij* plants are variegated and give rise to exceptional white seedlings in their progeny, even when they are pollinated by wild-type plants (Rhoades, 1943; Rhoades, 1946). Typically, from 1%-20% of the progeny inherit defective plastids in this way when *ij/ij* plants are used as the female parents, but no defective plastids are inherited when *ij/ij* plants are used as the male parents. This phenomenon has been a paradigm in cytoplasmic inheritance for more than 50 years (Goodenough, 1979).

The mechanism of uniparental cytoplasmic inheritance in higher plants is complex, and likely to involve degeneration of plastids and plastid DNA within the microspore, exclusion from the generative cell, expulsion from the sperm cells and exclusion from the zygote, as well as via asymmetric distribution of organelles in the egg (reviewed by Mogensen, 1996). Nonetheless, many flowering plants display biparental inheritance (*Pelargonium*, *Medicago* and *Oenothera*, for example) by which some or even most of the plastids found in the zygote come from the pollen. These mechanisms are thought to be under genetic control. For example in alfalfa, natural genotypic variants that are strong female transmitters have been found. In *Chlamydomonas*, mating-type specific transmission of plastids is controlled by a cluster of genes at the mating type locus, which control the destruction and protection of the plastids transmitted by each type of gamete, respectively (Armburst et al., 1993). By analogy, higher plant variants in these genes may fail to destroy plastids inherited through the male gametes.

We are using *ij/ij* plants to investigate the genetic basis for maternal inheritance in maize. We have constructed strains that are *ij/ij; bz-mum9/bzshwx; MuDR*. Pollen from these plants was mutagenized by highly active *Mutator* transposons, and used to generate 40,000 F1 seed with *bzshwx* testers. Gametophytic mutations that act in the pollen grain to allow transmission of defective plastids can be identified by examining the F1 seedling progeny for green-white variegation. Out of 30,000 F1 seedlings screened, 4 variegated plants were found, indicating that they had inherited white plastids from the *ij/ij* pollen parent. Two of these plants were partially male sterile, suggesting that they carried a defect in pollen formation that had been rescued by the vegetative nucleus in the previous generation. This showed that the appropriate

generation had been targetted by the mutagenesis, but these mutants may reflect problems in the last pollen mitosis rather than specific deficiencies in plastid transmission. The other two mutants are more-or-less fully fertile and may be more specific. All of the mutants have been propagated and will be retested for paternal transmission of defective plastids in the next generation.

Double mutants and modifiers. In maize, we have looked for genetic interactions of *iojap* with the modifier inhibitor of striate (*isr*). Since the work of R. A. Emerson in the 1930s, *Isr* has been recognized as a dose-dependent genetic modifier of variegation in leaf striping mutants of maize such as *striate2* (*sr2*). In the presence of *Isr*, *sr2* mutants have reduced leaf width concomitant with loss of white tissue. Leaf narrowing is due to loss of intermediate veins and ground tissue located at leaf margins, and the few remaining plastid defective cells are of irregular size and aberrant organization (Park et al., 2000). Similar narrowing of *ij* leaves is observed in certain inbred backgrounds, and we have begun to investigate the effect of *Isr* on *ij*. We have introgressed one, two or three copies of *Isr* into several inbred backgrounds carrying *ij*. So far, in the Tr and Oh51a backgrounds there is no effect of *isr* on the *iojap* phenotype. These backgrounds do not promote striping of *ij*, but rather have a uniform pale green/ grainy or albino phenotype. We are currently exploring the effect of *Isr* on variegated *ij* phenotypes found in the background W22.

Chang-deok Han, a former postdoc in the lab, has cloned the *Isr* gene by transposon tagging, in work he began at CSHL (Park et al., 2000). *Isr* mRNA is expressed throughout young leaves, but *Isr* chimaeras indicate that the expression of *Isr* at leaf margins is sufficient to suppress both the lateral expansion of *striate2* leaves and the extent of striping. *Isr* encodes a chloroplast protein with similarity to bacterial phosphatases involved in carbon catabolite repression or in carbon metabolism. There is a highly conserved *Isr* homolog in *Arabidopsis*. In collaboration with Dr. Han, we are currently generating monoclonal antibodies to the *Isr* protein in maize.