Kikudome found a comparable situation when he studied the effect of KlO on crossing over in a chromosome 9 bivalent heteromorphic for a knobless chromosome and a small, medium, or large knob. Crossing over increased from 26.9% to 31.5%, from 17.7% to 26.8%, and from 12.7% to 30.3%, respectively. These results represent increases of 17.1%, 51.5%, and 140%. It was suggested that there was an upper limit of about 30% recombination in the region investigated. In tests of Tp9Tp9 recombination there is a dosage effect of B chromosomes; therefore, the 33-34% commonly found with one B cannot be an upper limit.

Edward Ward


With few exceptions, the genetic information specifying the unique amino-acid sequence of an enzyme-subunit polypeptide is transmitted through meiosis as a single cistron. A diploid cell contains "one" paternal and "one" maternal allele for each gene, where the number "one" connotes one unit of genetically transmissible information (allele), as opposed to one unit of biosynthesis (here called "cistron"). Certain somatic cells might contain many redundant copies (cistrans) of one gene, copies which are transmitted through mitosis, without violating Mendelian laws. Even gametes may be redundant for any genetically single allele if one invokes the speculative "master-slave" relationship (Callan, 1960; 1967) among the redundant cistrans. Cytological and biochemical techniques presently available lack the resolution needed to detect small amounts of redundancy. This short note outlines a genetic strategy theoretically able to discover redundancies between zero and about twenty copies of a specific enzyme-specifying allele, and gives preliminary data for one of the alcohol dehydrogenase genes (Adh1; ADH enzyme, EC 1.1.1.1.) in maize root primordial cells. This strategy may have general applications.

Experimental strategy:

There are two alleles for each gene in a diploid somatic cell. If each allele were represented by but one cistron, then the maximum number of different polypeptides specified by a particular gene is two
per diploid cell. If, on the other hand, a number of cistrons represent each allele in a single cell, then a somatic mutation affecting one of the redundant cistrons could yield a single cell with three different polypeptides specified by only two genetically defined alleles. The following strategy may provide: (1) a way to detect relatively small amounts of a mutant polypeptide; (2) a control to ascertain whether that cistron specifying the mutant polypeptide was really in the same cell with both non-mutant parental alleles; and (3) a simple method by which a single somatic root primordial cell can be cloned within the living plant.

Mutant cistrons which specify enzyme subunits with an altered net surface charge can be detected by electrophoretic procedures. The Adh₁ gene of maize specifies product which is assembled into ADH enzyme dimers (Schwartz, 1966). A number of mutant and variant alleles exist for the Adh₁ gene (Schwartz, 1966). For example, Fig. 1 represents electrophoretograms—gels specifically stained for ADH activity—characterizing the three genotypes segregating on an Adh₁⁴⁴/ Adh₁⁴⁴ self-pollinated ear, where superscripts "S" and "F" denote alleles which specify subunits conferring a relatively "slower" or "faster" anodal electrophoretic migration rate to the ADH dimers containing these subunits (Schwartz, 1966). Most ADH dimers are stable in extracts and gels, and none has displayed the slightest tendency to dissociate and reassociate under standard in vitro conditions. The presence of a hybrid enzyme, marked by an arrow in Fig. 1, implies that there are at least two cistrons for the Adh₁ gene per cell, since there are at least two qualitatively different subunits synthesized. This conclusion is trivial. However, in the following test involving somatic mutation and clonal analysis, the presence of an "unexpected" hybrid enzyme may identify a somatically redundant allele.

Given a single somatic cell heterozygous for a normal Adh₁ allele (+) and an Adh₁ deletion or mutant allele specifying inactive, non-dimerizing polypeptides (−), it is possible to treat this +/− cell with a mutagen, wash out or discontinue treatment, allow clones to develop, and then electrophoretically analyze the clone. The reason for using an − mutant allele is to permit the analysis of but one Adh₁ allele of
Fig. 1. Three example electrophoretograms representing the three genotypes segregating on an Adh₁⁻/Adh₁⁻ self-pollinated ear. The arrow marks the "hybrid enzyme."

<table>
<thead>
<tr>
<th>Target Cell</th>
<th>Alternative Mutant Types</th>
<th>Enzyme Profile of Clone</th>
<th>Summary Interpretation, *see text</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>or</td>
<td></td>
<td>Non-mutant.</td>
</tr>
<tr>
<td>II</td>
<td>or</td>
<td></td>
<td>Supports, with one special exception, zero redundancy.</td>
</tr>
<tr>
<td>III</td>
<td>or</td>
<td></td>
<td>Unambiguous. Strongly supports somatic redundancy. Ratio between &quot;bands&quot; varies with number of redundant copies.</td>
</tr>
<tr>
<td>IV</td>
<td>or</td>
<td></td>
<td>Ambiguous. See &quot;preliminary data&quot; for detailed interpretation of this alternative.</td>
</tr>
<tr>
<td>V</td>
<td></td>
<td></td>
<td>Uninterpretable.</td>
</tr>
</tbody>
</table>

*All interpretations are based on the assumption that the "o" allele does not participate in the specification of any enzyme profile.

Fig. 2. Alternative types of ± allele mutations in a target cell as expressed in the enzyme profile of the mutant clone, with summary interpretations. See text for detailed interpretations and their bases.
the somatic cell. It is assumed that the rate of back-mutation or somatic crossover mediated o reactivation yielding a dimerizing subunit \((o \rightarrow + \text{ or } m)\) is negligible compared to the rate of forward mutation \((+ \rightarrow m)\) of the normal + allele.

Fig. 2 diagrams some of the alternative types of + allele mutations in a somatic target cell, as expressed in the enzyme profiles of the mutant clones. Interpretations assume that the o allele does not participate in the specification of these electrophoretograms. Alternatives II and III are most valuable, and ought to be mutually exclusive. Alternative II argues strongly against somatic redundancy since a single mutation \((+ \rightarrow m)\) changed all of the active ADH enzyme of the clone to a form with a new net surface charge, (but this can also be explained if a master \(\text{Adh}^*_1\) mutant allele corrects all slaves at each mitosis). In alternative III, one of a group of + cistrons must have mutated to a new cistron(m) specifying an active, dimerizing subunit with an altered surface charge. This alternative strongly supports somatic redundancy. The more + cistron copies extant in the mutated cell, the more difficult it becomes to detect a mutant cistron \((m)\), since the mutant ADH's would be a diminishing proportion of the total ADH specified. Whether the "clone" is actually a clone, or a mixture of mutant \((m/o)\) and non-mutant \((+o)\) cells certainly affects the feasibility of the test, but does not lead to erroneous interpretations. Alternative II requires a clone to be expressed; alternative III supports redundancy in a clone or in a cell mixture.

In order to realize the alternatives catalogued in Fig. 2, one demands a system where somatic "target cells" can be mutated and subsequently cloned until enough cells are present for electrophoretic assay. Some cells in culture are amenable to such analysis. The root system in higher plants may present a less obvious method for clonal analyses. Fig. 3 diagrams a possibility: some lateral roots, induced from the primary root by meristem excision, are clones, each of which develops from one and only one primordial cell in the embryo. If this hypothetical cell lineage were true for a reasonable proportion of laterals, mutually exclusive alternatives II and III would be detectable. In this case,
Fig. 3. Possible lineage of the cells of one lateral root from one "target cell" in the embryo of the seed.

Fig. 4. A representation of the electrophoretograms characterizing the two mutant-type lateral roots, with explanations (2) and (3) diagramed. Explanation (2): mixture of mutant and non-mutant cells. Explanation (3): somatic redundancy, here drawn as unineme for convenience only. See text for details.
seeds are treated with a mutagen and lateral roots are electrophoretically assayed. As mentioned previously, if lateral roots do not develop as suggested the experiment will fail, but will not lead to erroneous conclusions.

It is hoped that this experimental strategy might be generally applied in somatic mutation studies, and might serve as a test for gene redundancy.

Preliminary data:

Seeds of the \(+/o\) genotype were treated with ethyl-methanesulfonate, washed, dried and subsequently germinated. 543 lateral roots from 89 seedlings were electrophoretically analyzed following a brief anaerobic treatment to induce ADH activity. A known marker enzyme was present in every gel to exclude electrophoretic artifacts. Two independent, indistinguishable aberrant lateral roots were found, both corresponding to the ambiguous alternative IV of Fig. 2. The other 15 laterals from these two seedlings were normal. Fig. 4 diagrams what was seen in the aberrant electrophoretograms. Each of the following three explanations can account for these data. (1) The assumption on which this strategy is based is false: the new enzyme "band" is totally or in part the product of a reverted \(o\) allele. This unlikely explanation is being tested by analyzing somatic mutations of electrophoretically distinguishable \(+\) alleles in heterozygotes with the same \(o\) allele. (2) The aberrant lateral roots each contain two types of cells: \(+/o\), yielding the normal enzyme, and \(m/o\), specifying the new homodimer "band." In this case, both of the aberrant laterals must have had a very similar mixture of cells. (3) The lateral root primordial cell contains at least one \(o\) cistron, at least one \(+\) cistron, and at least one \(m\) cistron, the latter specifying either enzymatically active or inactive product which does dimerize. In this case, the new enzyme "band" is a hybrid enzyme, and somatic redundancy is supported; the \(m\)-\(m\) homodimer is not seen in the electrophoretograms either because it is inactive, or because many copies of the \(+\) cistron existed in the target primordial cell, or both. Fig. 4 gives a pictorial representation of explanations (2) and (3).
More mutants are needed to distinguish between these three explanations. Schwartz (published and unpublished data) has selected many ethyl-methane-sulfonate-induced Adh- alleles which are genetically transmissible and which specify subunits with altered net surface charges. These mutants specify certain charge transitions (e.g. the net loss of two negative charge units per subunit), but not others. The type of mutant required for explanation (2) to be correct has never been found, while that necessitated by explanation (3) is frequent.

Michael Freeling

In response to the interest expressed by a number of corn geneticists in kernel photography, the following article was contributed by the director of the Photographic Laboratory at Indiana University.

5. Kernel photography.

Close-up color photographs of corn kernels with clear reproduction of patterns of color variegation may be obtained in the following way. A camera of the single lens reflex type is used with Ektochrome high speed film. The lens used for close-up work is a 48 mm Micro-Tessar lens. A five inch extension tube may be used to increase magnification slightly. Lighting from above and below gives a white background; without the light from below, the background is black. Polarizing filters are of utmost importance for avoiding glare from the surface of the kernel. Filters are placed in front of the lens, as well as on each of the light sources. The highlight reflections on the kernel may be used for focusing; they are then removed by turning the polarizing filters before exposing the film. Dust particles on the kernels should be removed with a static-master brush.

C. M. Flaten