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Update section

Mini review

The importance of starch biosynthesis in the wrinkled seed shape character of peas studied by Mendel

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Abstract

The wrinkled-seed mutant (*rr*) of pea (*Pisum sativum* L.) arose through mutation of the gene encoding starch-branching enzyme isoform I (SBE1) by insertion of a transposon-like element into the coding sequence. Two isoforms of starch-branching enzyme have been documented in the developing pea embryo. The second isoform, SBEII, is expressed towards the later stages of embryo development while SBEI is expressed highly in the early stages. Due to mutation of SBEI the total amount of starch and the proportion of amylopectin, a branched starch polymer, are greatly reduced in the wrinkled (*rr*) line as compared to that in the wild-type, round (*RR*) line. Consequently, the level of sucrose in the *rr* line is nearly two fold that of the *RR* line. Increased sucrose concentration in the developing embryos of this mutant line causes increased uptake of water and thereby increases the cell size and fresh weight. During seed maturation in these mutant seeds a greater loss of water occurs. As a result, the wrinkled seed phenotype develops. Besides this morphological variation, the mutation also causes changes in the amount of lipid and of one storage protein, legumin. This review article discusses the role of the SBEI enzyme in causing such metabolic changes in the developing embryos with the implication that metabolism can play a central role in plant development.

Introduction

One of the seven characters of the pea plant studied by Gregor Mendel [27] to establish the general principles of genetics was the wrinkled versus round phenotype of mature seeds [4]. This character is governed by the *rugosus* (*r*) locus [42] which is located in chromosome 7 [17]. The wrinkled-seeded phenotype was described as early as the beginning of the 17th century [25]. Wrinkled seed also results from mutations at other loci including *rb* and *rug* 1, 2, 3 [24, 40]. However, these mutations were isolated relatively recently and all the wrinkled lines existing prior to this are believed to be due to a single mutation at *r* ([6], C. Martin, unpubl. results). Although the *r*, *rb* and *rug* mutations have the same external phenotype, *r* mutants can be uniquely distinguished by their starch granule morphology. The shape of starch granules from a wild-type (*RR*) seed is a simple oval, while that from a mutant (*rr*) seed is fissured and apparently compound [9, 18, 20, 21]. The amount of starch in most wrinkled-seeded lines is reduced compared to that of a wild-type round seed. Wrinkled seeds (*rr*) have about 50% less starch than wild-type seeds [24]. Starch granules are composed of two forms of starch polymers. They are amylose, a linear mol-

ecule, and amylopectin, a branched molecule. The percentage of amylose in the starch of wrinkled seeds (*rr*) is more than double that in the starch of round seeds (*RR*) [6]. This indicates impaired branching of polymers to form amylopectin in *rr* wrinkled seeds. Since this step of the pathway is catalysed by the starch-branching enzyme, it seemed likely that the *r* mutation might be a lesion in a gene encoding this enzyme [14, 15, 16, 26, 36], or, alternatively, that the *r* locus regulates the expression of starch-branching enzyme [6]. Consistent with these ideas, wrinkled (*rr*) embryos were found to have much lower activities of starch-branching enzyme during development than wild-type embryos [16, 26, 36].

At least some of the observed effects of the *r* mutation upon the seed might readily be explained by a deficiency in starch-branching enzyme during development. A lower rate of branching would provide fewer ends of glucose chains as substrates for starch synthase. This could lower the overall rate of starch synthesis and lead to an increase in sucrose, the primary carbon source for starch synthesis [16, 35, 36]. Consistent with this, wrinkled seeds have a higher sucrose content than round seeds and higher levels of metabolites on the pathway of starch synthesis from sucrose [14–16]. A high sucrose content, in turn, accounts for the wrinkled shape of the mature seed, since the increased osmotic potential of the embryo increases water uptake during seed development, swelling the mutant embryo to a larger size, and stretching its testa to a greater extent than the wild-type embryo. The testa then wrinkles due to the relatively greater decrease in volume as water is lost at the end of development.

Purification and cloning of starch-branching enzyme isoform 1

The observed deficiency in branching enzyme in the *r* mutant embryo, and the fact that this could potentially account for several aspects of the mutant phenotype, led us to study this enzyme in near-isogenic round- and wrinkled-seeded lines developed by Hedley *et al.* [20]. These lines confirmed earlier observations that starch quantity, quality and granule shape, as well as seed water content, are all affected by the *r* locus [40, 41]. Proteins with starch branching enzyme activity were purified from developing embryos of the wild-type [36]. Anion exchange chromatography of the highly purified enzyme separated the activity into two fractions. One was associated with a protein of about 114 kDa (SBEI) and the other was associated with a protein of about 100 kDa (SBEII). The purified enzyme from wrinkled embryos contained only the 100 kDa protein (SBEII). An antiserum raised against SBEI protein inhibited the activity of starch-branching enzyme in wild-type embryos and recognized a 114 kDa protein [6]. This protein was absent from wrinkled embryos. Screening of a cDNA library from developing embryos of the wild-type in the expression vector λgt11 using this antiserum yielded seven cDNA clones from the same transcript amongst 3×10^5 pfu. Sequence analysis of the longest cDNA clone revealed high similarity to the glycogen-branching enzyme of *Escherichia coli* at the amino acid level [6].

Characterization of the SBE1 gene and its identity as the *r* locus

Analysis of transcripts from the SBEI gene revealed that, although the SBEI protein is absent from *rr* embryos, a small amount of mRNA is produced. This transcript is about 800 nucleotides larger than that in wild-type embryos [6] (Fig. 1). Characterization of the SBEI gene in *rr* mutant embryos revealed that there is a transposon-like element of 800 bp in the coding sequence of the SBEI gene. This element shows similarities in its sequence to the Ac/Ds family of transposable elements in maize, in that it has caused an 8 bp target site duplication of the SBEI gene and has terminal inverted repeats with 8 out of 11 nucleotides identical to the terminal inverted repeats of Ac. Internally the element is extremely AT-rich and in this respect resembles closely the elements *Ds1* and *rUq* from maize. The increased size of the SBEI transcript in *rr* embryos is presumably

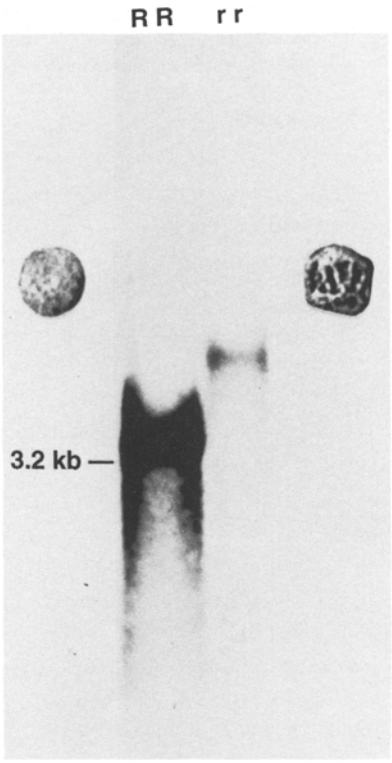

Fig. 1. Northern blot of RNA from embryos of *RR* and *rr* line. Poly(A)$^+$ RNA (10 μg) from embryos of *RR* and *rr* line was blotted and probed with the SBEI cDNA (pJSBE5, [6]).

due to transcriptional readthrough of these transposon sequences. Finally, linkage analysis of the SBEI gene in a population segregating for round and wrinkled seed established the location of the SBEI gene as the *r* locus of pea [6].

Expression of genes encoding SBEI and SBEII during development

Attempts to clone the second 100 kDa isoform of starch-branching enzyme SBEII using the SBEI cDNA clone as a probe were not successful, presumably because of low levels of identity at the nucleic-acid level between the genes encoding the two isoforms. Cloning of SBEII using an antiserum specific for this isoform revealed that the two isoforms are about 50% identical at the deduced amino acid level (R. Burton, D. Bewley, M. Bhattacharyya and C. Martin, unpubl. data). Analysis of transcript levels revealed that SBEI is expressed at maximum levels early in embryo development, whereas SBEII is maximally expressed considerably later (C. Martin, unpubl.). Consistent with this, starch branching enzyme activity in the *rr* mutant embryo lacking SBEI is negligible during the early part of development, but rises later [36].

Other mutations and wrinkled seeded phenotype

The mutation at the *rb* locus has very similar phenotypic effects to that at the *r* locus. The mature seed is wrinkled, the starch content is reduced and the sucrose levels are increased [24, 40]. The fact that the *r* mutation exerts all of its effects through reduction of the activity of an enzyme of starch synthesis suggested that the same might be true for the *rb* mutation. A survey of activities of enzymes on the pathway from sucrose to starch in wild-type and *rb* mutant embryos showed that the mutation dramatically reduced the activity of ADP glucose pyrophosphorylase. This plastidial enzyme catalyzes the synthesis of ADP glucose, the substrate for starch synthase. The mutation appears to eliminate one of its putative subunits [22, 34]. Although the nature of the gene(s) at the *rb* locus is not yet known, it seems very likely by analogy with the *r* mutation, that the mutant phenotype is attributable to the effect on ADP glucose pyrophosphorylase.

In addition to the spontaneous *r* and *rb* mutations that show the wrinkled seed phenotype, a series of pea mutants with wrinkled seeds have been obtained recently from a mutagenesis experiment on wild-type seed [39, 40]. These mutants were obtained by treating seeds with ethylmethanesulphonate or methylnitrosourea. On the basis of the seed composition, these mutants were classified into 8 groups. Two groups were allelic to *r* and *rb* respectively. Other groups are not allelic to either *r* or *rb* and have modified starch contents and amylose to amylopectin ratios. It will be interesting to learn about these mutations at the molecular level. New mutations at the *r*

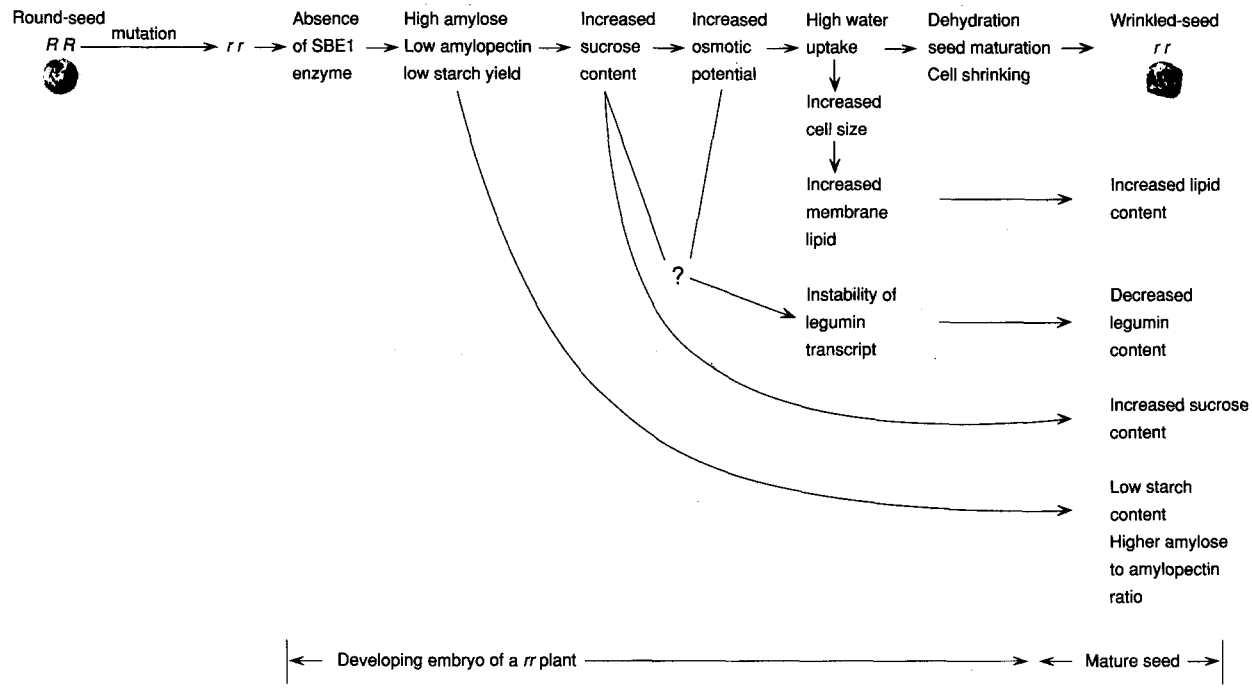

Fig. 2. Representation for the possible consequences arising from the mutation at the *r* locus.

locus ($R \rightarrow r$) i.e. in the SBEI gene are of special interest since they provide an allelic series to test the relative contribution of SBEI to starch biosynthesis and seed development. A series of mutants with different levels of SBEI activity provides material for measuring the flux control coefficient of the enzyme in starch biosynthesis and determining when this enzyme may limit starch production.

Wrinkled seed mutants are also recorded from other species. These include the *shrunken 1* (*sh1*), *shrunken 2* (*sh2*), *brittle 2* (*bt2*), *dull* (*du*) and *sugary* (*su*) mutants of maize [28, 29] and some of the Risø high-lysine mutants of barley [13, 33]. In all these mutants there is an elevated level of sucrose, and reduced starch content. Some of the mutants have been biochemically characterized. The best understood are those at the *sh2* and *bt2* loci. The genes at these loci have been shown to encode the two sorts of subunit which make up the ADP glucose pyrophosphorylase holoenzyme [2, 7]. Mutations which prevent the formation of a functional subunit dramatically reduce enzyme activity, and thus reduce starch content and increase sucrose content leading, in the same way as for pea seeds, to a wrinkled phenotype. Other cereal mutations affect the activities of one or more enzymes of starch synthesis, but are not understood at a molecular level.

Pleotropic effects of the *r* mutation through metabolic changes

The mutation at the *r* locus is associated with changes in levels not only of starch and sucrose, but also of lipids and a class of storage proteins, legumins (see [6]). Isolation and characterization of the *r* locus has shown that all these effects are metabolic consequences of altering starch biosynthesis and not due to any direct regulatory role of the *r* locus on structural genes on the pathways of synthesis of these products.

The higher lipid content of mutant embryos is due to increases in both storage and structural (membrane) lipids. It does not appear to reflect simply the increased availability of carbon resulting from reduced starch synthesis in these em-

bryos [5]. The whole structure of the embryo is affected by the *r* mutation, primarily because of the increased water content and hence larger volume. The average cell size of mutant embryos is greater than that of wild-type embryos [1]. Although the mechanism by which the lipid content of mutant embryos is increased is not known, it is likely that it reflects a greater membrane area in mutant than in wild-type embryos [5].

Levels of both protein and transcripts of legumin, one of two main classes of storage globulins in the embryo, are considerably reduced in *rr* mutant embryos, whereas levels of the other main class, vicilin, are not affected. Runoff transcription experiments indicate that the rates of transcription of legumin genes are the same in wild-type and *rr* mutant embryos, and it has been proposed that legumin transcripts may be selectively unstable at the higher osmotic potential of the mutant embryo [38]. Culture of wild-type embryos on media with increased sucrose contents and consequently internal osmotic potential also results in decreased levels of legumin protein and transcript, although there are only minor effects on vicilin [38]. An alternative interpretation may be that since sucrose and amino acid transport into developing embryos are linked, alterations in sucrose content of embryos may affect sucrose uptake, and hence amino acid uptake. Amino acid supply may be an important determinant of storage protein biosynthesis as has been shown for zein synthesis in maize [3]. A similar association between starch metabolism and protein synthesis has been documented for mutations affecting cereal seeds [12, 30, 37]. In maize association of reduced synthesis of the storage protein, zein, with reduced starch production in a number of *opaque* mutants [12] indicates a coupling of synthesis of storage protein and starch through what may be viewed primarily as a metabolic control of gene expression. It has also been suggested that many of the Risø mutants of barley, characterized by low levels of the storage protein hordein and of starch, may have their primary lesions in the starch biosynthetic pathway [33]. Similarly, in potato tuber, the accumulation of the storage protein patatin (class I) may be regulated by cellular sucrose levels or the rate of starch biosynthesis [32]. From the study with the *r* locus in pea and mutants of maize and barley it is apparent that mutations in the starch biosynthetic pathway play a major role in altering the amount of not only starch but also other seed storage products which are possibly regulated in their accumulation by a system that senses metabolite availability and hence determines final seed composition. Synthesis of seed storage products would appear to be controlled by the availability of sugars and amino acids, and their relative proportions may determine the partitioning into particular storage reserves. Some evidence suggests that this control operates on the expression of genes, either directly on expression of storage protein genes or on the expression of biosynthetic enzymes (e.g. for starch). This type of control responding to the metabolic status of the organism is likely to operate not only in developing embryos (as in peas) but also in other developing storage organs such as endosperm (as in cereals) and tubers (as in potato) reflecting their common function rather than their developmental origins.

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