Students have difficulty imagining how the genotype, operating at the primary level of organization to produce a polypeptide, can effect higher order phenotypic expression. One reason for the difficulty is that hierarchical complexity of cell, tissue, and organ system interaction is skirted when a genotype is correlated to a complex trait (as, for example, \( R = \text{round}, r = \text{wrinkled} \)). This exercise explores the biological basis for a classic genetic trait, round versus wrinkled peas, by investigating the multiple (pleiotropic) effects that the gene product, starch branching enzyme, has on metabolism, shape of the starch grain, and osmotic potential.

The \( RR \) seeds contain two forms of starch branching enzyme, one that activates early in seed formation and the other late. In wrinkled seeds, \( rr \), only the late acting form is present. Without the starch branching enzyme in early development, sugar precursors are converted to straight chain polysaccharides (amylose) rather than branched polysaccharides (amylopectin) by the enzyme starch synthase. Fewer branches means fewer sites to bond sugars to growing polysaccharides and, therefore, an accumulation of sugar molecules. This, in turn, increases osmotic pressure, increases water accumulation as the pea seed grows, and causes greater water loss when the pea dehydrates by the completion of development. A shriveled (wrinkled) seed results. Bhattacharyya et al. (1990) cloned the gene and discovered the \( r \) allele contains a 0.8 kb insertion of a transposable element not found in the \( R \) allele. The insertion alters the protein sequence.

Students are given dehydrated peas of three types (Early Alaska [EA], Thomas Laxton [TL], and Little Marvel [LM], available from Carolina Biological Supply Co.) and asked to determine which are round [EA] and which are wrinkled [TL, LM]. Several peas of each strain, hydrated in water overnight, are ground with mortar and pestle containing 10 ml of water. Wet mounts reveal that EA produces kidney bean-shaped starch grains; those of the other two strains resemble a sand dollar and are often fragmented. Extracts are centrifuged at 2500 rpm and the supernatant dropped onto agar plates prepared with glucose-1-phosphate (5 g G-1-p, 20 g agar, 1000 ml water). After 30 minutes, the plate is flooded with IKI and observed for starch production (positive for LM, TL) that reflects enhanced starch phosphorylase activity. The latter, which is not coded by the \( r \) gene, is likely an indirect metabolic effect of starch branching enzyme. Finally, students compare the wet and dry weight of peas to determine the percent difference in water content (highest for LM, TL).