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Tobacco Fraction 1 Protein: A Unique Genetic Marker

Synthesis of this photosynthetic enzyme is regulated by both the nuclear and chloroplast genomes.

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The discovery by Wildman and Bonner (*1*) in 1947 of a major protein in green leaves, designated as fraction 1 protein, forms the basis for some of the most recent advances in plant biology. Fraction 1 protein, comprising more than 50 percent of the soluble leaf protein, is the most abundant protein in nature and can be easily identified in aqueous extracts of leaves by its sedimentation coefficient (*2*). This protein is found in all organisms containing chlorophyll a, including the prokaryotic blue-green algae. Fraction 1 protein is identical to ribulose-1,5-diphosphate (RuDP) carboxylase-oxygenase (E.C. 4.1.1.39), a unique enzyme having dual functions that either fix or lead to the evolution of CO_2 (*3*). This enzyme catalyzes the crucial reactions of both photosynthesis and photorespiration. The ratio of these two processes ultimately determines the plant's productivity.

The concerted efforts of numerous groups of investigators in laboratories throughout the world have provided the current information on the structure, function, genetics, synthesis, and evolution of fraction 1 protein. This protein has also been successfully used as a genetic marker for probing many nucleocytoplasmic relationships in plants (*4*). The crystalline form of fraction 1 protein exhibits several curious properties (*5*). It is extremely soluble in the presence of RuDP, whereas in the absence of RuDP and in the presence of Mg^{2+} and HCO_3^-, the enzyme is extremely insoluble (*6*). This property provides the basis for the simplification of the crystallization procedure described by Chan *et al.* (*7*). Crystals are produced from leaf homogenates that have been centrifuged to remove particulate matter and passed through G-25 Sephadex to separate phenolic compounds. The yield is about 4 milligrams of crystals for each 1 gram of fresh leaf tissue (*8*). Recent results demonstrated that crystals can also be obtained from tobacco callus (*9*). The crystalline fraction 1 protein from tobacco contains no carbohydrate (*10*) and no metals (*11*).

Structure and Function

Fraction 1 protein is an 18*S* molecule, corresponding to the most commonly reported molecular weight of 5.6×10^5 (*2*). This suggests a high level of organization of the molecule. Fraction 1 protein can be dissociated into large and small subunits with molecular weights of 5.5×10^4 and 1.2×10^4, respectively, under a variety of denaturing conditions such as urea, sodium dodecyl sulfate, and extreme *p*H (*2*). On the basis of the molecular size and the ratio of the large and small subunits, as well as the shape of crystals, Kawashima and Wildman (*12*) proposed a molecular model for tobacco fraction 1 protein. They suggested that the native protein is constructed from eight large and six small subunits. Studies by Baker *et al.* (*13*) using combined information of x-ray diffraction data, electron micrographs, molecular weight, and the crystal density, demonstrated that the most likely structure of tobacco fraction 1 protein is eight large and eight small subunits (L_8S_8). Only in this structure can all binding domains between large and small subunits be equivalent. Therefore, the symmetry of this protein is a two-layered structure, with each layer consisting of four large and four small pairs. In fact, physical and chemical studies have already provided evidence for eight copies of large subunits (*12, 14*). The immunological evidence of Gray and Kekwick (*15*), who used French bean fraction 1 protein, demonstrated that the determinant groups on the isolated small subunit are not available at the surface of the native fraction 1 protein, suggesting that the small subunit may be buried within the protein with the large subunit at the surface.

Amino acid compositions (Table 1), tryptic peptide analyses (Fig. 1), and immunological comparisons (*16, 17*) show that the large subunit shares no similarities with the small subunit from the same species, but that large subunits from different species are very similar. Conversely, small subunits from different species are quite dissimilar. These data (Table 1) otherwise reveal no unique structural features. Fraction 1 protein from several plant species have approximately 95 half-cystine residues, all of which appear to be cysteinyl residues (*2, 18*).

This stability in composition of the large subunit is at least partly due to the fact that chloroplast DNA contains the code for arranging the sequence of about 500 amino acids in the primary structure (below). Genetically, chloroplast DNA is considered polyploid (*19*). Polyploidy is a conservative force in evolution. On the other hand, the variability in composition of the small subunit most probably is a consequence of the fact that nuclear genes

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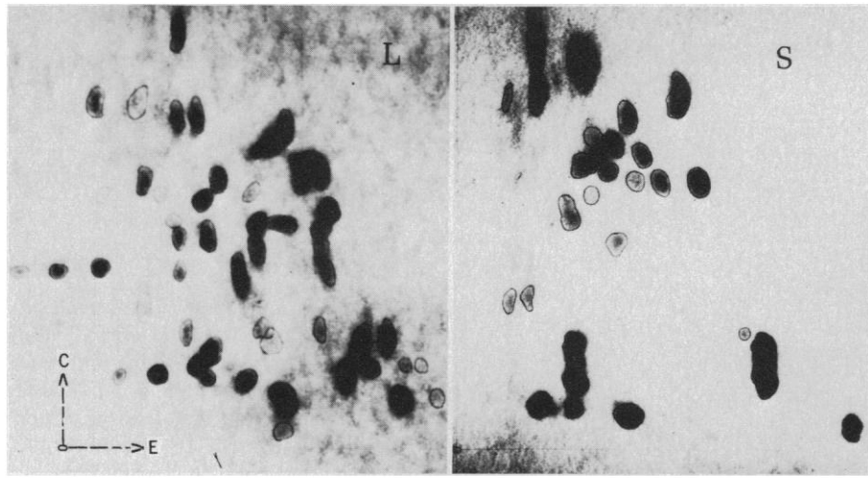

Fig. 1. Photographs of the peptide patterns of tryptic digests of the large (*L*) and small (*S*) subunits of *N. tabacum* fraction 1 protein. Origins are at the lower left corners. Solvent chromatography (*C*) was in the vertical direction; electrophoresis (*E*) was in the horizontal direction.

code for its primary structure and both paternal and maternal parents contribute to the genetic makeup of the primary structure of the small subunit (*4*).

Isoelectric focusing of *S*-carboxymethylated fraction 1 protein from *Nicotiana tabacum* in polyacrylamide gel has resolved the subunits into their component polypeptides (*20*). The eight large subunits were resolved into three polypeptides, each with a molecular weight of 55,000, and the eight small subunits were resolved into two polypeptides, each with a molecular weight of 12,500. The isoelectric points of the *S*-carboxymethylated large and small subunits are approximately *p*H 6.0 and 5.3, respectively (*20*). Examination of fraction 1 protein from 60 species of *Nicotiana* and ten plant species ranging from green algae to ginkgo reveals that all large subunits consist of three polypeptides whereas the small subunits may vary from one to four polypeptides (*4*). On the basis of the molecular weight and the arginine-lysine composition (*17*), more than 55 tryptic peptides would be obtained if the large subunit of *N. tabacum* contains three polypeptides of different primary structure but similar molecular weight. The fact that only 55 tryptic peptides were obtained from the isolated large subunit (Fig. 1) leads to the obvious question of the origin of these three polypeptides. They could be due to (i) three distinct polypeptides coded by three separate chloroplast DNA genes, or (ii) post-translational modifications, such as deamidation of glutaminyl or asparaginyl residues (*21*) of a single gene product producing three polypeptides of different charge. The two polypeptides of the small subunit of fraction 1 protein, however, can have their differences in composition detected by their two-dimensional chromatographic peptide patterns (*17, 20*).

Since fraction 1 protein comprises a major portion of the total soluble protein in leaf tissue, it was natural to assume that it plays an important role in cell metabolism. Dorner *et al.* (*22*) demonstrated that fraction 1 protein is identical to RuDP carboxylase. This enzyme is located in the stroma of chloroplasts where it catalyzes the CO_2 fixation step in photosynthesis. Recent evidence (*3*) indicates that fraction 1 protein also catalyzes an oxygenation reaction of RuDP, whose product (glycolic acid) is widely regarded as the primary substrate for photorespiration. Accordingly, this enzyme is now referred to as RuDP carboxylase-oxygenase and, therefore, may regulate both photosynthetic CO_2 fixation and photorespiratory CO_2 evolution. The evolutionary logic of the dual function for fraction 1 protein is not known. Tolbert (*3*) has suggested that simultaneous *p*-glycolate formation with CO_2 fixation may be an essential feature of photosynthesis, perhaps to concentrate CO_2 at the location of this enzyme. This is similar to the widely accepted concept that the C_4 dicarboxylic acid cycle functions for concentrating CO_2 (*23*).

Some studies have provided information not only on the dual function of this enzyme, but also the function of its subunits. The immunological studies of Gray and Kekwick (*15*), the reconstitution experiments of Nishimura *et al.* (*24*), and the work of McFadden on *Rhodospirillum rubrum* (*25*) demonstrate that the catalytic site of RuDP carboxylase-oxygenase activity is located in the large subunit. Several groups have proposed that the small subunits contain the regulatory sites (*15, 24*). Our observation of the genet-

Table 1. Amino acid composition of subunits obtained from different species of fraction 1 protein. The data are adapted from Kawashima and Wildman (*2*).

Amino acid	Large subunit				Small subunit			
	Sodium dodecyl sulfate–G-100			Urea–G-200	Sodium dodecyl sulfate–G-100			Urea–G-200
	Spinach	Spinach	Tobacco	Spinach beet	Spinach	Spinach	Tobacco	Spinach beet
Phenylalanine	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Lysine	1.18	1.04	1.07	1.15	1.21	1.19	1.77	1.44
Histidine	0.67	0.66	0.62	0.62	0.45	0.53	0.42	0.21
Arginine	1.44	1.42	1.44	1.26	0.99	0.96	0.96	0.58
Asparate	2.18	2.35	2.27	2.00	2.14	2.31	2.88	1.44
Threonine	1.75	1.63	1.45	1.41	1.19	1.29	1.60	0.77
Serine	0.81	0.87	0.86	0.93	0.77	0.78	1.51	0.91
Glutamate	2.20	2.42	2.52	2.30	2.26	2.77	4.14	2.17
Proline	1.13	1.17	1.13	1.06	1.56	1.56	1.64	1.43
Glycine	2.10	2.43	2.44	2.22	1.15	1.28	2.33	1.58
Alanine	2.16	2.21	2.23	2.13	0.86	0.98	1.63	1.09
Valine		1.52	1.49	1.57		1.15	1.36	1.40
Methionine	0.42	0.35	0.36	0.33	0.46	0.43	0.41	0.30
Isoleucine	0.88	0.69	0.76	0.88	0.56	0.47	0.84	0.70
Leucine	2.09	2.19	2.16	1.91	1.63	1.81	2.10	1.54
Tyrosine	0.92	0.91	0.85	0.76	1.57	1.43	1.92	0.99

ic control on the RuDP carboxylase-oxygenase activity with a tobacco mutant (*26*) provides direct evidence in support of this proposal. The effect of this nuclear mutation can only alter the primary structure of the small subunit (as discussed below). Therefore, differences in the RuDP carboxylase-oxygenase activity of the mutant and wild-type plants are a direct reflection of the regulatory function of the small subunit (Table 2).

Genetics, Synthesis, and Regulation

Advances made in localizing the genetic information of fraction 1 protein are mainly based on interspecific hybridization studies of the genus *Nicotiana*. The basis for using the *Nicotiana* system is that some heritable factors affecting the structure of chloroplasts are transmitted from generation to generation only by the maternal line (*4*). Therefore, the reciprocal hybrids (hybrids in which the sources of male and female gametes are reversed) provide information for localizing the coding message of fraction 1 protein on either the chloroplast or nuclear DNA. If the genetic information for a chloroplast protein can be transmitted through both the maternal and paternal lines, then it is likely that this information is located on chromosomal DNA residing in the nucleus. Conversely, if the mode of inheritance is strictly maternal, the coding information is contained in chloroplast DNA.

Figure 2 presents a photograph of parallel electrofocusing of fraction 1 protein of *N. excelsior*, *N. gossei*, *N. glauca*, *N. tabacum*, and the reciprocal hybrids between *N. glauca* × *N. tabacum*. It illustrates that the large subunit of fraction 1 protein from all four species consists of three polypeptides, whereas the small subunits contain one to four polypeptides. In the case of *N. glauca* and *N. tabacum*, one of the three large subunit polypeptides of *N. glauca* protein has a different isoelectric point from those of *N. tabacum* protein. This difference reflects the difference in amino acid composition between the large subunits of these two species (*17*). In the reciprocal hybrids, the isoelectric points of the three large subunit polypeptides correspond to the isoelectric points of the polypeptides of the female parent. The three large subunit polypeptides are inherited and expressed together; there is no separation of the inheritance of the individual polypeptides. This genetic analysis obtained by electrofocusing is entirely consistent with the genetic analysis of the tryptic peptides of the isolated large subunits from different species (*27*). Both of these methods demonstrate that the genes which

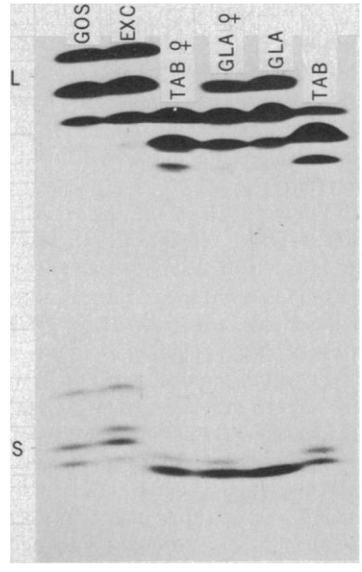

Fig. 2. Genetic analysis of *Nicotiana* fraction 1 protein by electrofocusing. The polypeptide composition of large (*L*) and small (*S*) subunits of fraction 1 protein from (left to right) *N. gossei* (*GOS*), *N. excelsior* (*EXC*), *N. tabacum* ♀ × *N. glauca* ♂ (*TAB* ♀), *N. glauca* ♀ × *N. tabacum* ♂ (*GLA* ♀), *N. glauca* (*GLA*) and *N. tabacum* (*TAB*). The large subunits consist of three polypeptides and a small subunit of one to four polypeptides.

code for the large subunit are inherited only from the maternal line and are therefore located in chloroplast DNA (*27*).

Genetic analysis of the coding information for small subunits of fraction 1 protein also is illustrated in Fig. 2. The polypeptides at the bottom of the gel (Fig. 2) belong to the small subunit of fraction 1 protein. The small subunit of *N. glauca* protein is composed of a single polypeptide, whereas the small subunit of *N. tabacum* consists of two polypeptides with different isoelectric points. This result is consistent with the difference in amino acid composition of the small subunits between these two species (*17*). The small subunit polypeptide characteristic of both parents is found in each of the reciprocal, *N. glauca* × *N. tabacum* F_1 hybrids. Therefore, each hybrid contains genetic information for the small subunit that was brought to the female parent by pollen and is, thus, contained in nuclear DNA (*27*).

The evidence for the site of synthesis of subunits of fraction 1 protein was first provided by studies with inhibitors. Criddle *et al.* (*28*) reported that chloramphenicol specifically inhibited the synthesis of the large subunit, whereas cycloheximide preferentially inhibited the synthesis of the small subunit. They suggested that the large subunit of fraction 1 protein from barley was synthesized within the chloroplasts and the small subunit was synthesized at a separate site, probably in the cytoplasm. Such a conclusion was not totally convincing because of the uncertainties of the mode of action of inhibitors such as chloramphenicol and cycloheximide in plant tissues (*29*). They could disrupt cellular metabolism in ways other than by inhibiting protein synthesis. This problem has been resolved by using cell-free systems and immunological approaches in place of inhibitors. Blair and Ellis (*30*) showed that intact, isolated pea chloroplasts, when driven by light energy, incorporated labeled amino acids into the large subunit of fraction 1 protein, the only soluble polypeptide to be synthesized. Hartley *et al.* (*31*) have shown, with a cell-free *Escherichia coli* preparation as the heterologous protein-synthesizing system, that the large subunit of fraction 1 protein is synthesized when total spinach chloroplast RNA is presented as the message. Gooding *et al.* (*32*), using wheat seedlings, and Gray and Kekwick (*15*), using bean plants, independently reported that cytoplasmic ribosomes (80*S*), active in synthesizing the small subunit, were preferentially precipitated by the antiserum to the small subunit. These results (*15, 30–32*) therefore suggest that cytoplasmic ribosomes make the small subunit of fraction 1 proteins, while chloroplast ribosomes (70*S*) make the large subunit.

It is now clearly established that the large subunit is coded for by the chloroplast genome; its messenger RNA (mRNA) is presented in chloroplast RNA, and it is synthesized on chloroplast ribosomes. The small subunit is coded for by the nuclear genome; its mRNA is present in cytoplasmic RNA, and it is synthesized on cytoplasmic ribosomes.

Since separate coding information as well as different site of synthesis are re-

Table 2. Differences in RuDP carboxylase-oxygenase activities of fraction 1 protein of the wild-type and yellow mutant leaves of John Williams Broadleaf, a cultivar of *N. tabacum* (*26*).

Fraction 1 protein	Carboxylase activity (10^2 count/min per mg protein)	Relative carboxylase activity (%)	Oxygenase activity (nmole O_2 per mg protein per min)	Relative oxygenase activity (%)
Wild-type (*su/su*)	414.3	100	25.8	100
Mutant (*Su/su*)	236.5	57	10.1	39
Mixture (*su/su*: *Su/su*) (1 : 1 mixture)	317.2	76	17.0	66

quired for fraction 1 protein, some control mechanisms must be exerted in such a fashion that the synthesis of large and small subunits are coordinated. Ellis (*33*) has put forward an intriguing hypothesis for the controlling mechanism of the synthesis of this protein. 2(-4-Methyl-2,6-dinitroanilino)- *N*- methyl- proprionamide (MDMP), a specific inhibitor of initiation on 80*S* ribosomes, inhibits the synthesis of both the large and small subunit when applied to intact pea leaves. However, MDMP does not inhibit protein synthesis in chloroplasts. This result is consistent with the possibility that the small subunit acts as a positive factor required for the initiation of the translation of the mRNA for the large subunit. If this were the case, it would imply that the nuclear genome is still the master controlling the overall rate of synthesis of fraction 1 protein.

A Genetic Marker and Its Application

Since the synthesis of fraction 1 protein requires the genetic information of both nuclear and chloroplast genomes, this protein becomes a useful genetic marker. A number of instances in which the unique properties of this protein have been employed to solve problems of biological interest are discussed below.

1) *Demonstration of the function of chloroplast DNA and cooperation between nuclear and chloroplast genomes.* The maternal inheritance of some properties of chloroplast led to the vigorous search for chloroplast DNA. It is now well established that chloroplasts contain their own DNA (*34*). With the use of the *Nicotiana* system and the electrofocusing method, it was found that chloroplast DNA contains the coding information for the large subunit of tobacco fraction 1 protein. This has been demonstrated by analysis of the following reciprocal hybrids: *N. tabacum* × *N. glauca*, *N. tabacum* × *N. glutinosa*, *N. tabacum* × *N. sylvestris*, *N. tabacum* × *N. gossei*, and *N. glauca* × *N. langsdorffii*. These findings provide direct evidence in illustrating the genetic function of higher plant chloroplast DNA.

The synthesis of this vital and abundant protein requires the close cooperation and coordination of both chloroplast and nuclear genomes and of both cytoplasmic and chloroplast ribosomes. The large subunit is both encoded and synthesized within the chloroplast, while the small subunit is both encoded and synthesized outside the chloroplast. Although no evidence is available as to the precise site of assemblage of both subunits into fraction 1 protein (probably in the chloroplasts), the biological implication is nevertheless clear that there is a close intergenomic cooperation and integration. This system presents one of the clearer depictions of the interaction between an organelle and a nucleocytoplasmic system.

2) *Confirmation of the nature of parasexual hybrids.* Significant progress has been made in developing in vitro techniques for studying genetic transformation and the intergeneric somatic hybrid (*35*). Two methods are being explored for transferring genetic information in somatic plant cells: protoplast fusion and DNA-mediated transformation. The success of such experiments relies heavily on the availability of genetic markers. Fraction 1 protein has proved to be such a marker for identifying both chloroplast and nuclear gene products. The value of this protein as a genetic marker was illustrated by its use in confirming the nature of the parasexual hybrid plants produced by fusion of protoplasts of *N. glauca* and *N. langsdorffii*, as well as transfer of *N. suaveolens* chloroplasts to *N. tabacum* protoplasts (*36*). The expression of both nuclear and chloroplast genomes in these parasexual hybrid plants was examined by an analysis of the polypeptide composition of fraction 1 protein. Figure 3 shows the polypeptide composition of the large and small subunits of fraction 1 protein prepared from the leaves of the parasexual hybrid of *N. glauca* and *N. langsdorffii*, produced by protoplast fusion.

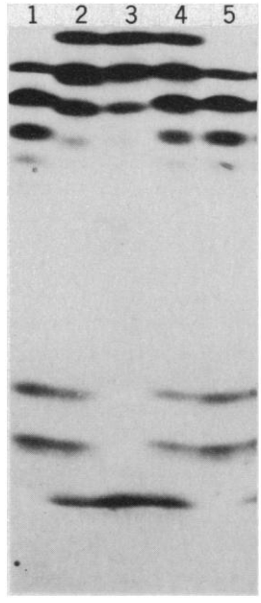

Fig. 3. Polypeptide composition of fraction 1 protein prepared from the parasexual hybrid of *N. glauca* and *N. langsdorffii*. (Channel 1), *N. langsdorffii*; (channel 2), parasexual hybrid of *N. glauca* and *N. langsdorffii*; (channel 3), *N. glauca*; (channel 4), equal mixture of *N. glauca* and *N. langsdorffii* protein recrystallized together; (channel 5), *N. langsdorffii*.

This is compared with the two parental species and an artificial mixture of the parental proteins. The results show that nuclear genes for the small subunits of both species are equally expressed in this parasexual hybrid, whereas only the chloroplast genome for the large subunit of *N. glauca* is expressed. The chloroplast transplantation experiments demonstrate that chloroplast DNA's from both parent plants are present and expressed in the hybrid plant. These experiments provide the first example that two genetically distinct populations of chloroplasts can live and function in a hybrid plant and they show that cell culture technology can be utilized to alter the genetic makeup of higher plants.

Another approach for achieving genetic variability is the construction of chimeral plants. Chimeral plant production has been achieved by Carlson (*37*) who induced plant formation in mixed calli from different plant species. Our preliminary results obtained from an analysis of fraction 1 protein of the chimeral plant leaves supplied by Carlson revealed that it contains the fraction 1 protein polypeptide composition from both plant species.

3) *Analysis of the origin, evolution, and speciation of the genus* Nicotiana. *Nicotiana tabacum* is the commercial tobacco plant. Since there is no well-authenticated record of its occurrence in the wild state, its origin and evolution are of interest. *Nicotiana tabacum* ($N = 24$) is believed to have arisen by chromosome doubling after hybridization of *N. sylvestris* ($N = 12$) females with either *N. otophora* ($N = 12$) or *N. tomentosiformis* ($N = 12$) as the male parent. Although genetic and biochemical (*38*) studies favor *N. tomentosiformis* as the male parent, the evidence is not conclusive. From an analysis by electrofocusing of fraction 1 protein isolated from *N. tabacum* and the putative progenitor species, it was confirmed that *N. tabacum* arose from the hybridization of *N. sylvestris* females with *N. tomentosiformis* males (*39*). Comparison with the polypeptide compositions of fraction 1 proteins from the above three species indicates that *N. sylvestris* contributed the large subunit polypeptides and, therefore, was the maternal parent in the original hybridization. *Nicotiana sylvestris* also contributed one of the two small subunit polypeptides of *N. tabacum*; the other polypeptide was contributed by *N. tomentosiformis*. This analysis indicates that the original hybridization was *N. sylvestris* females with *N. tomentosiformis* males and that *N. otophora* was not involved in the origin of *N. tabacum*.

The analysis of fraction 1 protein, therefore, provides a convenient means for de-

termining the origin of plant species that have arisen by interspecific hybridization. The particular advantage in the use of fraction 1 protein is that it is possible to determine the exact parentage in the original hybridization because the large subunit polypeptides are inherited solely from the maternal parent. Moreover, the method used with the *Nicotiana* species now has been successfully applied in the study of origin of wheat (*40*).

After the successful determination of the origin of *N. tabacum*, an attempt was made to understand how the polypeptide composition of fraction 1 protein evolves during the origin of new species of *Nicotiana*.

The 60 or more species of *Nicotiana* arose in nature primarily by hybridization between species (*41*). The F_1 hybrids were sterile because of the failure of the two complements of parental chromosomes to pair properly during meiosis. However, on extremely rare occasions, spontaneous doubling of the F_1 hybrid chromosomes, which permitted satisfactory pairing and subsequent fertility, occurred. This fertility was essential for a new species of *Nicotiana* to be capable of self-perpetuation by seeds.

The analysis of fraction 1 protein by electrofocusing shows that the polypeptide composition of this protein in the synthetic species, *N. digluta*, which arose spontaneously by doubling chromosomes in a population of sterile F_1 hybrids of *N. glutinosa* × *N. tabacum* (*41*), is identical to that of the F_1 hybrid, *N. glutinosa* females × *N. tabacum* males. The large subunit polypeptides are the same as those of *N. glutinosa*, as is required by the maternal inheritance of chloroplast DNA genes. The small subunit is composed of four polypeptides, two from *N. glutinosa* and two from *N. tabacum*, indicating an equal contribution of maternal and paternal nuclear genes (*42*). Thus, the two small subunit polypeptides of each of the parent species have been perpetuated in the new species to produce a fraction 1 protein with four small subunit polypeptides. These results are consistent with the proposed mechanism by which new fraction 1 protein evolves during the evolution of a new species of *Nicotiana*.

It has been shown that *N. tabacum* arose after interspecific hybridization of *N. sylvestris* × *N. tomentosiformis*, each of which has a single small subunit polypeptide, and that *N. digluta* arose from hybridization of *N. glutinosa* × *N. tabacum*, each of which has two small subunit polypeptides (*42*). Thus, hybridization of two species each with a single small subunit polypeptide could give rise to a new species with two small subunit polypeptides and a second round of interspecific hybridization could give rise to fraction 1 protein with four small subunits. Fraction 1 proteins with three small subunit polypeptides, as is found in some species of *Nicotiana*, could also have evolved from two hybridizations between *Nicotiana* species with one of the species in the second hybridization containing a single small subunit polypeptide.

An examination of the polypeptide composition of fraction 1 protein from many species of *Nicotiana* reveals a close correlation between the number of small subunit polypeptides and the ploidy of the species (*43*). Present-day species of *Nicotiana* may be subdivided into two groups on the basis of chromosome number, the 12-paired species and the 24-paired species. Fraction 1 protein from all 24-paired species contains two to four small subunit polypeptides. Most 12-paired species contain a single polypeptide. However, certain 12-paired species—for example, *N. glutinosa*—contain two small subunit polypeptides, which suggests that they may have originated by hybridization between two 6-paired species. There are no present-day 6-paired species in the genus *Nicotiana*, but Goodspeed (*41*) has suggested a 6-12-24-paired sequence as the basic evolutionary process in the genus.

4) *Elucidation of the origin of male sterile line.* Male sterility, characterized by the failure of the plant to produce viable or functional pollen, is a phenomenon widely distributed in the plant kingdom, particularly in cultivated plants. It is of unusual interest in cultivated plants not only because of the different causes that can give rise to it, but because of its intrinsic value to the plant breeder. The male sterile line analyzed was a Burley 21 cultivar of *N. tabacum*. The flowers were characterized by the complete absence of anthers, but could be fertilized by pollen from normal fertile Burley 21 plants. Analysis of fraction 1 protein from this male sterile cultivar revealed that it produced an isoelectric pattern of the two small subunit polypeptides identical to those for normal fertile Burley 21 plants. However, the isoelectric points of the three large subunit polypeptides were identical to those of another species, *N. suaveolens* (*43*). Thus, it was clear from our results and it was subsequently confirmed from the breeding records that the male sterile plants had been derived from an original cross between *N. suaveolens* females × *N. tabacum* males. Although the F_1 hybrid is male sterile, the ova can still be fertilized by nuclei in *N. tabacum* pollen and thus by repeated backcrossing and continued introduction of *N. tabacum* nuclear genes for the small subunit of fraction 1 protein; the *N. suaveolens* small subunit polypeptides were completely eliminated and replaced by the two *N. tabacum* polypeptides.

There are several important consequences of this analysis of the fraction 1 protein in the male sterile cultivar. (i) It demonstrates that in male sterile lines the chloroplast genome cannot be altered by conventional breeding techniques. (ii) It indicates that in studies on the physiological basis of male sterility greater emphasis ought to be placed on the effects of the chloroplast genome, as well as on the mitochondrial genome, which has been traditionally regarded as the source of the cytoplasmic factor in male sterility (*44*).

5) *Clarification of the source of tobacco disease resistance.* In the early 1930's a program was initiated to develop a root knot resistant tobacco. After 25 years of intensive studies, a resistant variety of *N. tabacum*, NC95, was finally released in 1960 (*45*). This variety was recorded to have been derived from a cross between a root knot resistant line (RK42) and a supposedly alloploid hybrid of *N. sylvestris* × *N. tomentosiformis*. The alloploid used was Kostoff's hybrid. The reported source of the resistance in NC95 is T1706, a progenitor of RK42. However, recent results indicated that the *N. sylvestris*, *N. tomentosiformis*, Kostoff's hybrid, and T1706 are very susceptible to this root knot disease (*46*). What was most surprising in these results was that another wild species of tobacco, *N. tomentosa*, had identical resistance reactions to this root knot disease as did NC95. It was then suggested that the originally employed Kostoff's hybrid may have had *N. tomentosa* as the male parent. A search of the breeding record of this particular hybrid revealed that Kostoff did cross *N. sylvestris* with both *N. tomentosiformis* and *N. tomentosa*.

Our analysis of the polypeptide composition of fraction 1 protein from all the species and hybrids involved in the breeding program revealed that both *N. tomentosiformis* and *N. tomentosa* have identical patterns: three polypeptides for large and one for small subunits. Therefore, a cross between *N. sylvestris* and *N. tomentosiformis* or *N. tomentosa* gives the same polypeptide composition as that of NC95 (*47*). This, together with the disease resistance results, suggests convincingly that the male parent in Kostoff's hybrid used for the original crossing with RK42 was *N. tomentosa* and not *N. tomentosiformis*, as had been reported. Our results demonstrate that it is the *N. tomentosa*, not T1706, that is the source of disease resistance.

Summary

Fraction 1 protein is found in all organisms that contain chlorophyll a, including the prokaryotic blue-green algae and is identical to ribulose-1,5-diphosphate (RuDP) carboxylase-oxygenase. This enzyme has dual functions in catalyzing both carboxylation and oxygenation of RuDP. Therefore, it catalyzes the crucial reactions of both photosynthesis and photorespiration; the ratio of these two processes will determine the plant's productivity.

Fraction 1 protein has a molecular weight of 560,000 and consists of eight large and eight small subunits arranged into a two-layered structure, each layer consisting of four large and four small subunits. The large subunit, with a molecular weight of 55,000, contains the catalytic site of the enzyme whereas the small subunit, molecular weight 12,500, is concerned with a regulatory function.

Studies with the *Nicotiana* and cell-free systems have shown that chloroplast genes contain the genetic information for the large subunit, whereas nuclear genes code for the small subunit. Immunological evidence also demonstrated that cytoplasmic ribosomes (80*S*) make the small subunit of fraction 1 protein, while chloroplast ribosomes (70*S*) make the large subunit. It has been suggested that the small subunit acts as a positive factor required for the initiation of the translation of the mRNA for the large subunit, implying that the nuclear genome is controlling the overall rate of synthesis of fraction 1 protein.

Recently, isoelectric focusing of *S*-carboxymethylated fraction 1 protein from *N. tabacum* in polyacrylamide gel has resolved the subunits into their component polypeptides. The eight large subunits were resolved into three polypeptides, each having a molecular weight of 55,000, and the eight small subunits were resolved into two polypeptides, each having a molecular weight of 12,500. Examination of fraction 1 protein from 60 species of *Nicotiana* and ten plant species ranging from green algae to ginkgo reveals that all large subunits consist of three polypeptides whereas the small subunits may vary from one to four polypeptides. The three polypeptides of the large subunit are inherited separately in a Mendelian fashion. This property of fraction 1 protein provides us with a genetic marker for both chloroplast and nuclear genomes. Consequently, it has been successfully used as a genetic marker for probing several biological problems of general interest. For example, this protein has been used as a genetic marker to (i) demonstrate the function of chloroplast DNA and cooperation between nuclear and chloroplast genomes; (ii) confirm the nature of parasexual hybrids; (iii) analyze the origin, evolution, and speciation of the genus *Nicotiana*; (iv) elucidate the origin of male sterile line; and (v) clarify the source of disease resistance in tobacco.

Our extensive knowledge of the fraction 1 protein is the product of fundamental research by several groups of scientists located in different institutions in the United States, Great Britain, Japan, Australia, and New Zealand. Collectively, they provide the current information on the structure, function, genetics, synthesis, regulation, and evolution of this protein.

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