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Plant Gene Register

Sequence of a Tobacco (*Nicotiana tabacum*) Gene Coding for Type A Phytochrome[1]

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Light is one of the most important environmental stimuli in the life of higher plants. Light is used not only as the source of energy for photosynthesis but also as an environmental signal for plant growth and development. To perceive the ever-changing spatial direction, quantity, and quality of light, higher plants developed several photoreceptors, among which the best studied and characterized is phytochrome. This unique plant chromoprotein exists as a dimer of two polypeptides of approximately 120 kD, each with a covalently attached linear tetrapyrrol chromophore (Vierstra et al., 1984).

The phytochrome molecule is synthesized as the physiologically inactive Pr form. The Pr form can be converted by red illumination (L_{max} 660–670 nm) to the active Pfr form, which can be returned to the inactive Pr form by a subsequent far-red illumination (L_{max} 730 nm). This photo-induced interconvertibility enables phytochrome to act as a cellular switch that controls signal transduction chains, culminating in different molecular and physiological responses; for a review see Furuya (1989).

There is evidence that plants contain more than one type of phytochrome (Tokuhisa et al., 1985). These different phytochrome polypeptides (called types A, B, C, and E) are encoded by a group of related but divergent *phy* genes (Sharrock and Quail, 1989). We are interested in characterizing the phytochrome system in tobacco (*Nicotiana tabacum*), which is widely used as a transgenic organism, to study light-regulated reactions in higher plants. As a first step toward this goal we isolated several cDNA and genomic clones encoding the type A phytochrome of *N. tabacum*.

Here we report the nucleotide and deduced amino acid sequences of the tobacco *Nt-Phy-A1* gene (Table I). The deduced protein shows 76, 83, and 92% identity with the type A phytochrome of *Arabidopsis thaliana*, pea, and potato, respectively (Tomizawa et al., 1986; Sharrock and Quail, 1989; Heyer and Gatz, 1992). Because *N. tabacum* is an allotetraploid species, its genome is assumed to contain at least two genes encoding type A phytochrome. We note that we have also isolated another type of tobacco genomic and cDNA clones that clearly encode type A phytochrome. One of these clones, designated *Nt-Phy-A2*, has been partially characterized. The 5′ region of the *Nt-Phy-A2* clone that

Table I. *Characteristics of Nt-Phy-A1 genomic clone*

Organism:
Nicotiana tabacum L. var NK 326.
Source:
Genomic library in EMBL3 vector (purchased from Stratagene).
Method of Identification:
The genomic library was screened with radiolabeled fragments of a 3200-bp cDNA. This cDNA clone was identified by screening a tobacco seedling cDNA library with a synthetic 60-mer oligonucleotide as probe. (This sequence spans the chromophore-binding region of the oat phytochrome cDNA clone, AP3, coding for type A phytochrome; Hershey et al., 1988.) Other techniques included restriction enzyme mapping, sequencing both strands of the subcloned fragments by dideoxy chain termination method (Sanger et al., 1977), and computer analysis.
Features of Gene Structure:
This clone contains a 960-bp 5′-untranslated region, a 4263-bp coding region, and a 717-bp 3′-untranslated region. The gene is interrupted by four introns, bounded by intron/exon splice junctions consistent with the consensus sequence (Brown, 1986) for such junctions. The size of the deduced *Nt-Phy-A1* (4488 nucleotides) shows good correlation with the *Nt-Phy-A1* transcript (4500 nucleotides) detected on northern blots.
Codon Usage:
The start codon is ATG. Codons not present: TAG, TAA.
(G+C) Content:
44. 37% in the coding region.
Structural Features of Protein:
The deduced protein contains 1125 amino acids and shows high (76–92%) similarity to type A phytochrome characterized in other plant species.
Antibodies:
Not available. Cross-reaction is expected with antibodies raised against pea and oat type A phytochrome (Furuya, 1989).

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spans the 5′-untranslated leader and the first intron shows 89% homology with the corresponding region of the *Nt-Phy-A1* gene. These data, together with the results of Southern hybridization experiments and the sequence analysis of the isolated cDNA clones, clearly show that the *N. tabacum* genome contains two genes encoding type A phytochrome.

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LITERATURE CITED

Brown JWS (1986) A catalogue of splice junctions and putative branch sequences from plant introns. Nucleic Acids Res **14:** 9549–9559

Furuya M (1989) Molecular properties and biogenesis of phytochrome I and II. Adv Biophys **25:** 133–167

Hershey HP, Barker RF, Idler KB, Lissemore JL, Quail PH (1988) Analysis and genomic sequences for phytochrome: amino acid sequences for two gene products expressed in etiolated *Avena*. Nucleic Acids Res **13:** 8543–8559

Heyer A, Gatz C (1992) Isolation and characterisation of a cDNA clone coding for potato *typeA* phytochrome. Plant Mol Biol **18:** 535–544

Sanger F, Nicklen S, Coulsen AR (1977) DNA sequencing with chain-termination inhibitors. Proc Natl Acad Sci USA **74:** 5463–5467

Sharrock RA, Quail PH (1989) Novel phytochrome sequences in *Arabidopsis thaliana*: structure, evolution and differential expression of a plant regulatory receptor family. Genes Dev **3:** 1745–1757

Tokuhisa JG, Quail PH (1985) Phytochrome in green tissue: spectral and immunochemical evidence for two distinct molecular species of phytochrome in light grown *Avena sativa*. Planta **164:** 521–528

Tomizawa KI, Komeda Y, Sato N, Nagatani A, Iino T, Furuya M (1986) Isolation of cDNA for pea phytochrome using an expression vector. Plant Cell Physiol **27:** 1101–1108

Vierstra RD, Cordonnier M-M, Pratt LH, Quail PH (1984) Native phytochrome: immunoblot analysis of relative molecular mass and *in vivo* proteolytic degradation for several plant species. Planta **160:** 521–528