Molecular Characterization of the Salutaridinol 7-O-Acetyltransferase Involved in Morphine Biosynthesis in Opium Poppy Papaver somniferum*

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Salutaridinol 7-O-acetyltransferase (EC 2.3.1.150) catalyzes the conversion of the phenanthrene alkaloid salutaridinol to salutaridinol-7-O-acetate, the immediate precursor of thebaine along the morphine biosynthetic pathway. We have isolated a cDNA clone that corresponds to the internal amino acid sequences of the native enzyme purified from a cell suspension culture of opium poppy Papaver somniferum. The recombinant enzyme acetylated the 7-hydroxyl moiety of salutaridinol in the presence of acetyl-CoA. The apparent Kₐ value for salutaridinol was determined to be 9 μM and 54 μM for acetyl-CoA. The gene transcript was detected in extracts from Papaver orientale and Papaver bracteatum in addition to P. somniferum. Genomic DNA gel blot analysis indicated that there is likely a single copy of this gene in the P. somniferum genome. The amino acid sequence of salutaridinol 7-O-acetyltransferase is most similar (37% identity) to that of deacetylvindoline acetyltransferase of Catharanthus roseus. Salutaridinol 7-O-acetyltransferase is the second enzyme specific to morphine biosynthesis for which we have isolated a cDNA. Taken together with the other cDNAs cloned encoding norcoclaurine 6-O-methyltransferase, (S)-N-methylcoclaurine 3'-hydroxylase, the cytochrome P-450 reductase, and codeine reductase, significant progress has been made toward accumulating genes of this pathway to enable the end goal of a biotechnological production of morphinan alkaloids.

The opium poppy Papaver somniferum produces some of the most widely used medicinal alkaloids. The narcotic analgesic morphine and the antitussive and narcotic analgesic codeine are the most important physiologically active alkaloids from this plant. Nineteen total syntheses of morphine have been reported through 1999 (1). The most efficient synthesis of morphine proceeded on medium scale with an overall yield of 29% (2). Despite many years of excellent synthetic organic chemistry concentrated on morphinan alkaloids, the plant appears to be related to the pentacyclic morphinan ring system (8).

Each of the known enzymes of morphine biosynthesis has been detected in both P. somniferum plants and cell suspension culture, yet plant cell cultures have never been shown to accumulate morphine or codeine (3). Morphine accumulation in the plant appears to be related to differentiation of a latex system (12). In efforts aimed at the metabolic engineering of the P. somniferum alkaloid profile as well as at developing alternate biotechnological sources of morphinans, we searched first to identify suitable genetic targets.

We report herein the molecular characterization of salutaridinol 7-O-acetyltransferase of morphine biosynthesis in P. somniferum. The native enzyme was purified to apparent homogeneity, amino acid sequences were determined for internal peptides, and a cDNA clone was generated by RT-PCR using

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank/EMBL Data Bank with accession number(s) AF339913.

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been almost completely elucidated by M. H. Zank and co-workers and is summarized by Kutchan (3). Morphine is derived from two molecules of the amino acid L-tyrosine in a series of at least 17 enzymatic steps. The latter steps in the pathway that lead specifically from (S)-reticuline, a central intermediate of isoquinoline alkaloid biosynthesis, to morphine involve three NADPH-dependent oxidoreductases (4–6), most probably three cytochromes P-450 (7), and an acetyl-CoA-dependent acetyltransferase (8).

Acetyl-CoA-dependent acetyltransferases have an important role in plant alkaloid metabolism. They are involved in the synthesis of monoterpenoid indole alkaloids in medicinal plant species such as Rauwolfia serpentina. In this plant the enzyme vinorine synthase transfers an acetyl group from acetyl-CoA to 16-epi-vellosin to form vinorine. This acetyl transfer is accompanied by a concomitant skeletal rearrangement from the sarpagin to the ajmalane type (9). An acetyl-CoA-dependent acetyltransferase also participates in vindoline biosynthesis in Catharanthus roseus, the source of the chemotherapeutic dimeric indole alkaloid vinblastine (10, 11). Acetyl-CoA:deacetyl-vindoline 4-O-acetyltransferase catalyzes the last step in vindoline biosynthesis. Central to morphine biosynthesis in P. somniferum is acetyl-CoA:salutaridinol 7-O-acetyltransferase (EC 2.3.1.150) (Fig. 1). Acetylation of the phenanthrene salutaridinol is followed by alllylic syn-displacement of the acetylated (activated) hydroxyl by the phenolic hydroxyl, which follows stereocontrol for SN2 substitution of cyclohexene rings, thereby producing the pentacyclic morphinan ring system (8).

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1 The abbreviations used are: RT-PCR, reverse transcriptase-polymerase chain reaction; HPLC, high performance liquid chromatography; RACE, rapid amplification of cDNA ends; MS, mass spectrometry; LC, liquid chromatography; salAT, cDNA encoding salutaridinol 7-O-acetyltransferase; TLC, thin layer chromatography; CAPS, 3-(cylohexylamino)-1-propanesulfonic acid; cor1, cDNA encoding codeine reductase; cyp80b1, cDNA encoding (S)-N-methylcoclaurine 3’-hydroxylase; DEPC, diethylpyrocarbonate; bp, base pairs.
P. somniferum mRNA as template. Heterologous expression in a baculovirus vector in insect cells yielded functional enzyme, and enzymic properties were determined for the recombinant protein. This is the second gene specific to morphine biosynthesis that has been isolated and characterized (13).

**EXPERIMENTAL PROCEDURES**

**Plant Material**—Cultured suspension cells of opium poppy P. somniferum were provided by the cell culture laboratories of the Lehrstuhl für Pharmazeutische Biologie and the Leibniz-Institut für Pflanzenbiochemie. Cultures were routinely grown in 1-liter conical flasks containing 400 ml of Linsmaier-Skoog medium (14) on 7 days at 23 °C on a gyratory shaker (100 rpm) in diffuse light (750 lux). Differentiated P. somniferum, Papaver bracteatum, Papaver orientale, Papaver nuditcaule, Papaver atlanticum, Papaver rhoeas, and Chelidonium majus plants were grown outdoors in Upper Bavaria or in Saxony-Anhalt, Germany. P. somniferum ssp. setigerum plants were grown in a greenhouse at 24 °C with 18 h of light and 50% humidity.

**Purification of Native Enzyme and Amino Acid Sequence Analysis**—Salutaridinol acetyltransferase was purified from P. somniferum cell suspension cultures exactly according to Lenz and Zenk (8). The purified enzyme preparation was subjected to SDS-polyacrylamide gel electrophoresis to remove traces of impurities, and the Coomassie Brilliant Blue R-250-visualized band representing the acetyltransferase was digested in situ with endoproteinase Lys-C as reported previously (15, 16). The peptide mixture was resolved by reversed phase HPLC (column, Merck Lichrospher RP18; 5 μm (4 × 125 mm); solvent system, 0.1% trifluoroacetic acid (A) and 0.1% trifluoroacetic acid, 60% acetonitrile (B); gradient, 1% per min; flow rate, 1 ml min−1; detection at 206 nm). Microsequencing of 10 of the peptides was accomplished on an Applied Biosystems model 470 gas-phase sequencer.

**Generation of Partial cDNAs from P. somniferum**—Partial cDNAs encoding salutaridinol acetyltransferase from P. somniferum were produced by PCR using cDNA generated by reverse transcription of mRNA isolated from 7-day-old suspension-cultured cells. DNA amplification using either Taq or Pfu polymerase was performed under the following conditions: 3 min at 94 °C, 35 cycles of 94 °C, 30 s; 60 °C, 30 s; 72 °C, 1 min. At the end of 35 cycles, the reaction mixtures were incubated for an additional 7 min at 72 °C prior to cooling to 4 °C. The amplified DNA was resolved by agarose gel electrophoresis, and the bands of approximately the correct size (537 bp) were isolated and subcloned into pGEM-T Easy (Promega) prior to nucleotide sequence determination. The specific sequences of the oligodeoxynucleotide primers used are given under “Results.”

**Generation of Full-length cDNAs—**The sequence information requisite to the generation of a full-length cDNA was derived from the nucleotide sequence of the partial cDNA produced as described under “Results.” The complete nucleotide sequence was generated in two steps using one salutaridinol acetyltransferase-specific PCR primer (5′-GCC GCA GGC CAA CAA GGG TTG AGG TGG-3′ for 5′-RACE and 5′-CCC ATC CTG CAC GAG CTA CTT ATC C-3′ for 3′-RACE) and one RACE-specific primer as specified by the manufacturer. The 5′- and 3′-RACE-PCR experiments were carried out using a Marathon cDNA amplification kit (CLONTECH). RACE-PCR was performed using the following PCR cycle: 3 min at 94 °C, 35 cycles of 94 °C, 30 s; 60 °C, 30 s; 72 °C, 2 min. At the end of 35 cycles, the reaction mixtures were incubated for an additional 7 min at 72 °C prior to cooling to 4 °C. The amplified DNA was resolved by agarose gel electrophoresis, and the bands of the expected size (1265 bp for 5′-RACE and 917 bp for 3′-RACE) were isolated and subcloned into pGEM-T Easy prior to sequencing.

The full-length clone was generated in one piece using the primers 5′-CCA TGG CAA CAA TGT ATA GTG CTG CTG-3′ and 5′-AGA TCG AAT TCA ATA TCA CAA TTC TGG AGG-3′ for PCR with P. somniferum cell suspension culture cDNA as template. The final primers used for cDNA amplification contained recognition sites for the restriction endonucleases NcoI and EcoRI appropriate for subcloning into pFastBac HTA (Life Technologies, Inc.) for functional expression. DNA amplification was performed under the following conditions: 3 min at 94 °C, 35 cycles of 94 °C, 30 s; 60 °C, 30 s; 72 °C, 2 min. At the end of 35 cycles, the reaction mixtures were incubated for an additional 7 min at 72 °C prior to cooling to 4 °C. The amplified DNA was resolved by...
agarose gel electrophoresis, and the band of the approximately correct size (1440 bp) was isolated and subcloned into pCR4-TOPO (Invitrogen) prior to nucleotide sequence determination.

**Heterologous Expression and Enzyme Purification** — The full-length cDNA generated by RT-PCR was ligated into pFastBac HTa that had been digested with restriction endonucleases NcoI and EcoRI. The recombinant plasmid was transposed into baculovirus DNA in the *Escherichia coli* strain DH10BAC (Life Technologies, Inc.) and then transfected into *Spodoptera frugiperda* Sf9 cells according to the manufacturer’s instructions. The insect cells were propagated, and the recombinant virus was amplified according to Kutchan *et al.* (17) and Pauli and Kutchan (18). INSECT-XPRESS serum-free medium (BioWhittaker) was used in the enzyme expression experiments. After infection of 150 ml of suspension grown insect cells had proceeded for 3–4 days at 28 °C and 130 rpm, the cells were harvested by centrifugation under sterile conditions at 1000 × g for 10 min at 4 °C. All subsequent steps were performed at 4 °C. The pellet was discarded, and the medium was slowly brought to 80% saturation with ammonium sulfate under constant slow stirring. The precipitated proteins were collected by centrifugation at 10,000 × g for 30 min at 4 °C. The pellet was dissolved in a minimal volume of 0.5 M NaCl, 10 mM β-mercaptoethanol, 2.5 mM imidazole, 20 mM Tris-HCl adjusted finally to pH 7.0 and was dialyzed for 12–16 h against this same buffer. The His-tagged salutaridinol acetyltransferase was purified by affinity chromatography using a cobalt resin (Talon, CLONTECH) according to the manufacturer’s instructions.

**Enzyme Assay and Product Identification** — The acetylation catalyzed by salutaridinol acetyltransferase was assayed according to Lenz and Zenk (8). The reaction mixture was extracted once with 1 volume of CHCl3 and was resolved by TLC (plates, silica gel 60 F 254, Merck; solvent system, chloroform:acetone:diethylamine (5:4:1)). The radioactivity present on the TLC plates was localized and quantitated with a Rita Star TLC scanner (Raytest). The identity of the enzymic reaction product as thebaine was ascertained by HPLC-MS using a Finnigan MAT TSQ 7000 (electrospray voltage, 4.5 kV; capillary temperature, 220 °C; carrier gas, N2) coupled to a Micro-Tech Ultra-Plus Micro-LC equipped with an Ultrasep RP18 column (5 μm, 110 mm). The solvent system was 99.8% (v/v) H2O, 0.2% HOAc (A) and 99.8% CH3CN (v/v), 0.2% HOAc (B) with a gradient of 0–15 min 10–90% B, 15–25 min 90% B and a flow rate of 70 μl min−1. The positive ion electrospray mass spectrum for thebaine (retention time, 17.4 ± 0.1 min; *m/z* = 312) was characteristic of the standard reference compound.

**FIG. 2.** Amino acid sequence comparison of salutaridinol 7-O-acetyltransferase to other plant acetyltransferases involved in secondary metabolism. **SALAT,** salutaridinol 7-O-acetyltransferase from *P. somniferum* (this work); **DAT,** deacetylvindoline acetyltransferase of *C. roseus* (22); **BEAT,** benzylalcohol acetyltransferase from *C. breweri* (26); **HCBT,** anthranilate N-hydroxycinnamoyl/benzoyltransferase from *D. caryophyllus* (27); **DBAT,** 10-deacetylbaccatin III-10-O-acetyltransferase and **TAT,** taxadienol acetyltransferase, both from *T. cuspidata* (28, 29). **Black boxes** indicate conserved residues; **white boxes** indicate the internal peptide sequences obtained from native salutaridinol 7-O-acetyltransferase; **arrows** indicate the positions of the peptides used to design oligodeoxynucleotide primers for RT-PCR; # denotes positions of the highly conserved consensus sequence LXXDX.
Fig. 3. Genomic DNA gel blot analysis of the salutaridinol 7-O-acetyltransferase gene in opium poppy. Genomic DNA isolated from P. somniferum 3-week-old seedlings was hybridized to salAT full-length cDNA and was visualized by phosphorimagery. The number of restriction endonuclease recognition sites that occur within the open reading frame are as follows: EcoRI, 0; HindIII, 0; Apol, 1; SalI, 1; SpeI, 1; HindII, 1; and MspI, 3. kb, kilobase pairs.

General Methods—Latex was collected and resolved as described previously (19, 20). Low molecular weight compounds were removed from the supernatant of the resolved latex by passage through a PD 10 column (Amersham Pharmacia Biotech) into 20 mM Tris, 10 mM β-mercaptoethanol, pH 7.5. Total RNA was isolated, and RNA gels were run and blotted as described previously (18). Genomic DNA was isolated, and DNA gels were run and blotted according to Decker et al. (21). cDNA clones were labeled by PCR labeling with [32P]dATP. Hybridized RNA on RNA gel blots and DNA on DNA gel blots were visualized with a STORM PhosphorImager (Molecular Dynamics). The entire nucleotide sequence on both DNA strands of the full-length clone was determined by dideoxy cycle sequencing using internal DNA sequences for the design of deoxyligonomucleotides as sequencing primers. Saturation curves and double reciprocal plots were constructed with the Program (Version 2.7, Biosoft, Cambridge, United Kingdom). The influence of pH on enzyme activity was monitored in sodium citrate- (pH 6–7.5), Tris-HCl- (pH 7.5–9), glycine/NaOH- (pH 9–10.5), and CAPS-buffered (pH 10–12) solutions.

RESULTS

Purification and Amino Acid Sequence Analysis of Salutaridinol 7-O-Acetyltransferase—Salutaridinol 7-O-acetyltransferase was purified to apparent electrophoretic homogeneity from opium poppy cell suspension cultures, and the amino acid sequence of 10 endoproteinase Lys-C-generated peptides was determined. The sequences and relative positions of these internal peptides are indicated by unshaded boxes in Fig. 2. A comparison of these amino acid sequences with those available in the GenBankTM/EMBL sequence data bases indicated no relevant similarity to known proteins. PCR primer pairs based on a series of salutaridinol 7-O-acetyltransferase peptide combinations also yielded only DNA fragments of irrelevant sequence.

Isolation of the cDNA Encoding Salutaridinol 7-O-Acetyltransferase—During the course of the initial RT-PCR experiments, sequence comparison information appeared in the literature for another acetyltransferase of plant alkaloid biosynthesis (22). The translation of the sequence of the cDNA encoding deacetylvindoline 4-O-acetyltransferase was homologous to a series of other putative plant acetyltransferases. A conserved region near the carboxyl terminus of the proteins was used to design a degenerate antisense oligodeoxynucleotide primer for PCR. The sense primer was based upon an internal peptide sequence of salutar-
The primer sequences were as follows: sense primer (FVDFFAK), 5'-TT(T/C) GT(G/A/T) TT(T/C) GA(C/T) TT(T/C) GC(A/T) AA-3'; antisense primer (DFGWG motif), 5'-A(C/G/T)GG (C/T)TT (A/C/G/T)CC CCA (A/C/G/T)CC(G/ A)AA (A/G)TC-3'.

The positions of these peptides are indicated by arrows in Fig. 2. RT-PCR performed with this primer pair yielded a DNA product of the correct size and sequence for the opium poppy acetyltransferase. RACE-PCR was then used to generate the 5'- and 3'-portions of the cDNA using nondegenerate nucleotide sequence information provided from the original PCR product.

Sequence Analysis of salAT—Translation of the complete nucleotide sequence of salAT yielded a polypeptide of 474 amino acids containing no apparent signal peptide. This is consistent with the cytosolic localization of the enzyme activity (6). The enzyme activity is also operationally found to be associated with the cytosolic fraction of exuded latex. The salAT amino acid sequence contains residues conserved in other plant acetyltransferases as indicated by the black boxes in Fig. 2. The longest contiguous region of conserved amino acids are the five residues DFGWG near the carboxyl terminus that were used for primer design and are indicated by an arrow. Conserved histidine and aspartate residues (HXXXD, denoted by # in Fig. 2) thought to be involved in catalysis as characterized by x-ray crystallography for the bacterial enzymes chloramphenicol acetyltransferase and dihydrolipoamide acetyltransferase are also present in salutaridinol 7-O-acetyltransferase (23, 24).

Covalent modification of salutaridinol 7-O-acetyltransferase by treatment with diethylpyrocarbonate (DEPC) resulted in the inhibition of enzyme activity (50% inhibition at 3 mM DEPC and 92% inhibition at 5 mM DEPC) (25). The inactivation by 5 mM DEPC was reduced from 92% to 46% by preincubation of the enzyme with 30 mM acetyl-CoA.

The amino acid sequence of salutaridinol 7-O-acetyltransferase is most similar (37% identity) to that of deacetylvinindo-line acetyltransferase of C. roseus (22). Other similar plant acyltransferases involved in secondary metabolism are benzyl-
alcohol acetyltransferase from *Clarkia breweri* (34%) (26), anthranilate N-hydroxycinnamoyl/benzoyltransferase from *Dianthus Caryophyllus* (25%) (27), taxadienol acetyltransferase (24%), and 10-deacetylbaccatin III-10-O-acetyltransferase (22%) both from *Taxus cuspidata* (28, 29).

**Genomic DNA and Gene Expression Analysis**—A genomic DNA gel blot analysis of *salAT* in *P. somniferum* is presented in Fig. 3. The restriction endonucleases *Apo I, Sal I, Spe I*, and *Hind II* each recognized one hydrolysis site within the *salAT* open reading frame yielding two hybridizing bands on the Southern blot. There were no recognition sites for *Hind III* in the open reading frame. Correspondingly, only a single band hybridized, but it was of approximately one-half the predicted length. This indicates the possible presence of a small intron in the gene. Three recognition sites were present for *Msp I*, theoretically resulting in four hybridizing DNA fragments. Two hybridizing bands of predictable length should have been present, but two hybridizing bands were present on the gel blot indicating an intron, which contains an open reading frame yielding two hybridizing bands on the open reading frame. The identity of the clone was ascertained by catalytic activity of the functionally expressed recombinant enzyme. The results obtained by RACE-PCR indicated that the open reading frame obtained by RNA gel blot analysis. The calculated molecular mass of the enzyme is 52.6 kDa, which is consistent with the apparent molecular mass of 50 kDa determined by SDS-polyacrylamide gel electrophoresis (8).

**Acyltransferases of related amino acid sequence develop into a family of genes with integral roles in plant secondary metabolism. This has been suggested previously by De Luca (22). The amino acid sequence of salutaridinol 7-O-acetyltransferase is similar to acyltransferases involved in monoterpenoid indole alkaloid, phenylpropanoid conjugate, and diterpenoid formation similar to acyltransferases involved in monoterpenoid indole alkaloid biosynthesis (22–29) and serine/threonine protein kinases (30). Histidine and aspartate residues (20%–25%) are highly conserved as is a DFGWG motif near the catalytic terminus of the proteins. The equivalent histidine residue has been shown through site-directed mutagenesis or chemical modification to be essential for catalytic activity in other acyltransferases (30). Preincubation of the enzyme with acetyl-CoA partially protected a putative active site histidine residue from chemical inactivation of the enzyme.

**DISCUSSION**

We have isolated a full-length cDNA clone that encodes salutaridinol 7-O-acetyltransferase from cell suspension culture of opium poppy *P. somniferum*. An identical match was observed between the deduced and directly determined amino acid sequences of ten internal peptides distributed throughout the open reading frame. The identity of the clone was ascertained by catalytic activity of the functionally expressed recombinant enzyme. The results obtained by RACE-PCR indicated that the open reading frame is 1425 nucleotides long corresponding to 474 amino acids. These values correlate well to the transcript size obtained by RNA gel blot analysis. The calculated molecular mass of the enzyme is 52.6 kDa, which is consistent with the apparent molecular mass of 50 kDa determined by SDS-polyacrylamide gel electrophoresis (8).

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modification and resultant inactivation. A catalytic triad (Ser-His-Asp) as found in serine proteases and lipases has been postulated for other acyltransferases (30). The crystal structure of arylamine N-acyltransferase from Salmonella typhimurium indicates that a cysteine residue may be a component of the catalytic triad (Cys69, His107, Asp122) (31). The amino acid sequence of salutaridinol 7-O-acyltransferase contains both a conserved serine (Ser23) and a conserved cysteine (Cys152) suggesting that a catalytic triad could also be essential to enzyme activity in this family of plant acyltransferases. This consensus information should certainly also aid in the identification and isolation of additional members of this family that may be involved in other plant secondary pathways.

The site of morphine biosynthesis in P. somniferum is a long-standing question. We examined herein the expression pattern of salAT in P. somniferum. salAT was expressed in each major plant part analyzed—root, stem, leaf, and capsule. This corresponds to the detection of transcript of another morphine biosynthesis-specific gene, cor1, in each plant organ analyzed (12). Additionally, salutaridinol 7-O-acyltransferase and codeinone reductase enzyme activity have each been detected in the cytosolic fraction of isolated latex (12, 13). The gene cyp80b1 participates in (S)-reticuline biosynthesis occurring before a bifurcation in the biosynthetic pathway that leads to more than 80 isoquinoline alkaloids. cyp80b1 is, therefore, common to several biosynthetic pathways including morphine, sanguinarine, and noscapine. Transcript of cyp80b1 was also detected in all plant organs analyzed (12). Accumulation of morphinan alkaloids is thought to correlate with the appearance of laticifer cells in the developing plant and in differentiating plant cell culture (32, 33). A reticulated laticifer system associated with the vascular tissue is present through the aerial parts of the poppy plant. In roots, nonreticulated laticifers are present (34, 35). The localization of three genes of morphine biosynthesis, cyp80b1, salAT, and cor1, is thus far consistent with the assumption this biosynthesis is, at least in part, associated with laticifer cells. Interestingly, deacetylvindoline acetyltransferase has been localized to laticifer cells in aerial parts of C. roseus (36).

With even more morphine biosynthetic genes now in hand, we can begin to address the question of why only P. somniferum produces morphine, whereas other Papaver species such as P. rhoes, P. orientale, P. bracteatum, P. nudicaule, and P. atlanticum do not. salAT transcript was detected in RNA isolated from P. somniferum, P. orientale, and P. bracteatum but not in RNA from P. nudicaule and P. atlanticum. This is consistent with the expected distribution based upon accumulation of alkaloids having the morphinan nucleus in these species (i.e. morphine in P. somniferum, thebaine in P. bracteatum, and oripavine in P. orientale). This is in sharp contrast to those results obtained for cor1 transcript, which was detected also in Papaver species that are not known to accumulate codeine (12). The genes of alkaloid biosynthesis in P. somniferum will certainly continue to provide useful information on the molecular evolution of plant secondary metabolism in latex systems.

An important aspect of our molecular genetics experiments with P. somniferum is an eventual biotechnological production of thebaine, codeine, and morphine. We have isolated cDNAs encoding several enzymes of morphine biosynthesis. The first enzyme in the biosynthetic pathway for which we have isolated a cDNA is norcoclaurine 6-O-methyltransferase (37). The second enzyme is the cytochrome P-450-dependent monoxygenase (S)-N-methylcoclaurine 3'-hydroxylase (12, 18). These enzymes are common to the morphine, noscapine, and sanguinarine biosynthetic pathways. Specific to morphine biosynthesis are salutaridinol 7-O-acyltransferase (reported herein) and codeinone reductase, the penultimate enzyme of the morphine pathway that reduces codeinone to codeine (13). A cDNA encoding an enzyme involved generally in metabolism but essential to the activity of the cytochrome P-450-dependent monoxygenase, the cytochrome P-450 reductase has also been isolated (38). Each of the cDNAs has been functionally expressed in insect cell culture (S. frugiperda Sf9 cells) or in E. coli. An immediate application of these cDNAs is in the metabolic engineering of P. somniferum to obtain altered alkaloid profiles in the plant. A more long-term goal is a biomimetic synthesis of morphinan alkaloids combining chemically and enzymatically catalyzed steps. For this latter application, additional cDNAs encoding enzymes that mediate transformations occurring between (R)-reticuline and morphine still need to be isolated.

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