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iaglu, a Gene from *Zea mays* Involved in Conjugation of Growth Hormone Indole-3-Acetic Acid

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Plants contain most of the growth hormone indole-3-acetic acid (IAA) in conjugated forms believed to be inactive in promoting growth. The *iaglu* gene, which controls the first step in the biosynthesis of the IAA conjugates of *Zea mays*, encodes (uridine 5′-diphosphate-glucose:indol-3-ylacetyl)-β-D-glucosyl transferase. Protein synthesized by *Escherichia coli* that contained cloned 1-*O*-β-D-indol-3-ylacetyl-glucose complementary DNA (cDNA) was catalytically active. The predicted amino acid sequence of the cDNA was confirmed by amino-terminal sequencing of the purified enzyme. Homologous nucleotide sequences were found in all plants tested. The blockage or enhancement of *iaglu* expression may permit regulation of plant growth.

All plants examined thus far contain most of the growth hormone IAA in a conjugated, presumably inactive, form (*1–3*). Plants exhibit a growth response to applied free (unconjugated) IAA, and there is evidence that growth rate is a function of endogenous free IAA concentrations (*4*). These results indicate that growth is limited and controlled by the amount of free IAA. In contrast, the conjugates appear to serve functions other than growth promotion, such as IAA transport (*5*), protection of IAA against peroxidative attack (*6*), storage of IAA in seeds (*1*, *7*), and hormonal homeostasis (*4*). Both ester- and amide-linked IAA conjugates have been chemically characterized (*1*, *7*). Because of the rapidity of conjugate synthesis and hydrolysis in vivo, the function of IAA conjugates has been difficult to study (*2*). The ability to augment or impair IAA conjugation may lead to a better understanding of the physiology of hormone conjugation and to methods for control of plant growth. Therefore, we cloned the *iaglu* gene, which encodes the first step in the IAA conjugation pathway.

In corn (*Z. mays*), the pathway to the conjugates begins with the synthesis of 1-*O*-β-D-indol-3-ylacetyl-glucose (IAGlu) from uridine 5′-diphosphate-glucose (UDPG) and IAA, catalyzed by the enzyme IAGlu synthetase (UDPG:indol-3-ylacetyl)-β-D-glucosyl transferase (Fig. 1) (*8–10*).

$$\text{IAA} + \text{UDPG} \rightleftharpoons \text{IAGlu} + \text{UDP}$$

IAGlu is an acyl alkyl acetal, and its energetically unfavorable synthesis is followed by an energetically favorable transacylation of IAA from IAGlu to *myo*-inositol (Fig. 1) (*11*).

We used polyclonal antibody (*10*) purified by affinity chromatography to screen a cDNA expression library made from polyadenylated RNA extracted from W64A$^+$ inbred corn endosperm tissue collected 18 days after pollination. The library was constructed in a lambda ZAP II vector (Stratagene) (*12*). After amplification, it contained 4.2×10^8 plaque-forming units. Eight positive clones were identified from among 1.5×10^6 *E. coli* XL-1 Blue plaques containing isopropyl-β-D-thiogalactopyranoside (IPTG)–induced β-galactosidase fusion proteins with an immunoglobulin G secondary antibody conjugated to alkaline phosphatase (*13*). Positive cDNA inserts were excised with R408 helper phage and recircularized to generate subclones in the pBluescript SK$^-$ phagemid vector (*14*). Both strands of the largest insert [clone 3, 1731 base pairs (bp)] were sequenced (*15*). The sequenced clone carried an open reading frame of 1413 nucleotides that coded for 471 amino acids (Fig. 2) (*16*). The open reading frame was rich in G and C nucleotides (C + G = 69.7%).

A portion of the purified enzyme was chromatographed on a C_{18} 1-mm by 250-mm high-performance liquid chromatography (HPLC) column with 0.1% trifluoroacetic acid (TFA) as the solvent and a gradient of 90% v/v acetonitrile-water containing 0.85% TFA. Protein degradation occurs (*10*), but the single major peak was collected for amino acid sequencing of the NH_2-terminal end. The 18–amino acid sequence obtained was MAPXVLVVPF-

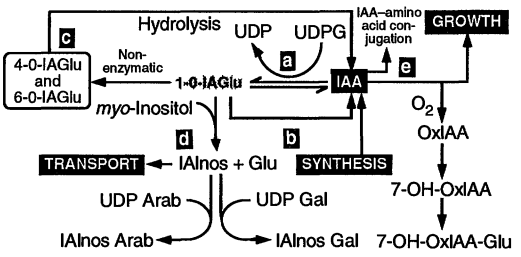

Fig. 1. Metabolic reactions that affect the concentration of IAA in *Z. mays*. (a) Reversible synthesis of 1-*O*-IAGlu from IAA and UDPG (*9*, *23*); (b) enzymatic hydrolysis of 1-*O*-IAGlu (*9*, *23*); (c) enzymatic hydrolysis of 4-O-, and 6-*O*-IAGlu produced by isomerization of 1-*O*-IAGlu (*23*, *24*); (d) transacylation of IAA from 1-*O*-IAGlu to form the transport ester, IAInos, and results in shifting the equilibrium toward esterified IAA (*7*, *8*, *11*); and (e) IAA may also be conjugated to amino acids (*1*, *7*).

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Fig. 2. Deduced amino acid sequence (*17*) of the cDNA sequence of the *iaglu* gene from *Z. mays* (Genome Sequence DataBase accession number L34847). Numbers at right indicate the amino acid position in the sequenced clone. The NH_2-terminal end of the deduced protein begins in position 1, and the underlined amino acids indicate amino-terminal end residues identified by sequencing the purified IAGlu synthetase. Asterisk denotes a stop codon.

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MAPHVLVVPFPGQGHMNPMVQFAKRLASKGVATTLVTTRFIQRTADVDAHPAMVEAISDGHDEGGFASAAGVAEY   75
LEKQAAAASASLASLVEARASSADAFTCVVYDSYEDWVLPVARRMGLPAVPFSTQSCAVSAVYYHFSQGRLAVPP  150
GAAADGSDGGAGAAALSEAFLGLPEMERSELPSFVFDHGPYPTIAMQAIKQFAHAGKDDWVLFNSFEELETEVLA  225
GLTKYLKARAIGPCVPLPTAGRTAGANGRITYGANLVKPEDACTKWLDTKPDRSVAYVSFGSLASLGNAQKEELA  300
RGLLAAGKPFLWVVRASDEHQVPRYLLAEATATGAAMVVPWCPQLDVLAHPAVGCFVTHCGWNSTLEALSFGVPM  375
VAMALWTDQPTNARNVELAWGAGVRARRDAGAGVFLRGEVERCVRAVMDGGEAASAARKAAGEWRDRARAAVAPG  450
GSSDRNLDEFVQFVRAGATEK*                                                         471
```

Fig. 3. Alignment of the predicted amino acid sequence (*17*) of the *iaglu* gene from *Z. mays* with selected UDPG- and UDP-glucoronic–glycosylating gene products. (**A**) Human *HlugP4* gene (*25*) showing a 68% sequence similarity (44% identity) over a stretch of 56 residues, and *Z. mays bz1* gene (Bz-McC allele) (*26*) showing 59% similarity (49% identity) over 131 residues. (**B**) An *Oryctolagus cuniculus UGT2B13* gene (*27*) showing 48% similarity (59% identity) over 52 residues, and a *Rattus norvegicus rlug23* gene (*28*) showing 55% similarity (44% identity) over 58 residues. Bars indicate identical amino acids, dots indicate similar residues (*29*), and numbers at left show residue number.

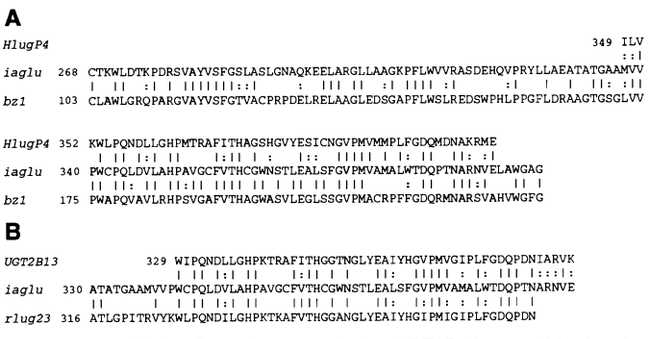
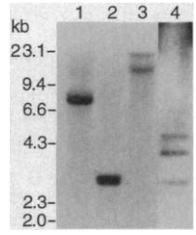


Fig. 4. An autoradiograph of Southern (DNA) blot hybridization of *Z. mays* and *A. thaliana* DNA with cDNA encoded for *Z. mays iaglu* gene. *Zea mays* genomic DNA (10 μg) was digested with Eco RI (lane 1), Hind III (lane 2), or Bam HI (lane 3). *Arabidopsis thaliana* genomic DNA (6 μg) was digested with Eco RI (lane 4). The filters were probed with *iaglu* cDNA radiolabeled with ^{32}P by random priming. High-stringency conditions were used for *Z. mays* DNA [hybridization at 2× standard saline citrate (SSC) at 65°C; last wash at 0.3× SSC at 65°C], and moderate-stringency conditions (hybridization at 4× SSC at 65°C; last wash at 1× SSC at 65°C) were used for the *A. thaliana* genomic DNA. Molecular size standards are shown at left.



PGQGXMNP (*17*), which corresponds exactly with the amino acid sequence of the NH_2-terminal end deduced from the nucleotide sequence of the isolated clone (Fig. 2). The two unknowns (X) in the 18-residue chain were shown to be histidines by the nucleotide sequence analysis. The calculated molecular size of the predicted protein was 49.71 kD and the estimated isoelectric point was 5.69, in agreement with determined values (*10*, *18*).

A computer search of DNA and protein sequence databases (*19*) revealed high homology of amino acids 268 to 393 of the *iaglu* gene with conserved domains of other UDPG- and UDP-glucuronic–glycosylating proteins (Fig. 3). Because all these proteins transfer either glucose or glucuronic acid to their specific acceptors, the conserved amino acids probably represent those necessary to bind UDP. A potential N-glycosylation site N-X-(S or T) (residue 363, Fig. 2) and three potential protein kinase C phosphorylation sites (S or T)-X-(R or K) have been identified (residues 37, 453, and 469) (Fig. 2).

The catalytic activity of protein synthesized by *E. coli* cells that contained the cDNA insert from clone 3 cloned into the Eco RI site of pBluescript SK$^-$ (*20*) was examined. Bacteria containing pBluescript SK$^-$ without an insert or bacteria containing a shorter, antibody-positive cDNA (clone 2, 1050 bp) were used as controls. Both controls did not result in the synthesis of labeled IAGlu. Clone 3 synthesized $^{13}C_6$-labeled IAGlu, which yielded $(M)^+ = 343.143$ and $(M + Na)^+ = 366.126$ when incubated with $^{13}C_6$-labeled IAA and UDPG, as determined by fast atom bombardment mass spectrometry. These masses confirm the elemental composition as $^{13}C_6C_{10}H_{19}O_7N$ and $^{13}C_6C_{10}H_{19}O_7NNa$, as expected for heavy-atom labeled IAGlu. Authentic, unlabeled IAGlu (*21*) yielded $(M)^+$ of 337.118 and $(M + Na)^+$ of 360.113, which corresponded to $C_{16}H_{19}O_7N$ and $C_{16}H_{19}O_7NNa$, respectively.

Organization of the *iaglu* gene in the *Z. mays* genome was studied with a radiolabeled *iaglu* cDNA sequence used as a probe (Fig. 4). At high-stringency conditions, a single Eco RI or Hind III fragment and two Bam HI fragments hybridized, which indicates that IAGlu synthetase is probably encoded by a single-copy or low–copy number gene in the *Z. mays* genome. In addition, genomic DNA from different plant species including *Arabidopsis thaliana*, tobacco, sugar beet (*Beta vulgaris*), tomato (*Lycopersicon esculentum*), soybean, cauliflower (*Brassica oleracea* variant *botrytis*), and duckweed (*Lemna gibba*) were analyzed (Fig. 4) (*22*). In all cases, at moderate stringency, multiple hybridizing bands (one major and several minor) were observed. This may reflect hybridization of the *Z. mays iaglu* cDNA probe to the equivalent *iaglu* genes of these plants, as well as to other genes encoding UDP-binding proteins. In *A. thaliana*, three hybridizing bands were observed with moderately stringent conditions. However, under high-stringency conditions, only a single, 3-kb hybridizing band was detected (*22*). The same single band was detected under moderately stringent conditions when the 5′-region of the *iaglu* cDNA (nucleotides 1 through 598, lacking the putative UDPG-binding site) was used as a probe, suggesting that this band contains the *A. thaliana iaglu* equivalent gene.

A small number of reactions appear to control the amount of growth hormone IAA in a plant. Thus, it may be feasible to genetically manipulate IAA concentrations and selectively control plant growth without the application of growth-regulating chemicals.

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15. The DNA sequence was determined by the dideoxy chain termination method with Sequenase Version 2.0 (United States Biochemical) and synthetic oligonucleotide primers. Compression of bands was elim-

inated by the use of 7-deaza deoxyguanosine 5′-triphosphate.
16. Analysis of the nucleic acid sequences and deduced amino acid sequence was by the computer program MacVector, release 3.5 (International Biotechnologies, New Haven, CT).
17. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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19. Computation of homology was performed by the BLAST Network Service (National Center for Biotechnology Information, Bethesda, MD).
20. The assay mixture for the determination of the enzymatic activity of the IAGlu synthetase gene cloned in pBluescript SK$^-$ (Stratagene) in *E. coli* was in a 0.5-ml final volume containing 0.8 mM $^{13}C_6$-labeled IAA and 0.05 μCi of [5-^{3}H]IAA (to facilitate IAGlu purification); 5 mM UDPG; 0.1 mM dithiothreitol; 75 mM Hepes buffer, pH 7.4; 50 mM *myo*-inositol; and 0.1 ml of enzyme extract (27 μg of protein). Incubation was for 4 hours at 37°C. After the sample was processed (*3*, *18*, *21*), the resulting material was scanned from 100 to 1200 daltons in a VG-ZAB2S mass spectrometer (VG-Analytical, Manchester, UK), proving the presence of $^{13}C_6$-labeled IAGlu.
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An Interleukin-4–Induced Transcription Factor: IL-4 Stat

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Interleukin-4 (IL-4) is an immunomodulatory cytokine secreted by activated T lymphocytes, basophils, and mast cells. It plays an important role in modulating the balance of T helper (Th) cell subsets, favoring expansion of the Th2 lineage relative to Th1. Imbalance of these T lymphocyte subsets has been implicated in immunological diseases including allergy, inflammation, and autoimmune disease. IL-4 may mediate its biological effects, at least in part, by activating a tyrosine-phosphorylated DNA binding protein. This protein has now been purified and its encoding gene cloned. Examination of the primary amino acid sequence of this protein indicates that it is a member of the signal transducers and activators of transcription (Stat) family of DNA binding proteins, hereby designated IL-4 Stat. Study of the inhibitory activities of phosphotyrosine-containing peptides derived from the intracellular domain of the IL-4 receptor provided evidence for direct coupling of receptor and transcription factor during the IL-4 Stat activation cycle. Such observations indicate that IL-4 Stat has the same functional domain for both receptor coupling and dimerization.

Studies of the mammalian immune system have led to the discovery of small, secreted proteins that provide circuitry to both dedicated and peripheral components of the system (1). These proteins, broadly termed cytokines, act to modify the growth and differentiated function of cells harboring cognate receptors. Cytokines are typically expressed under tight regulation with respect to cell type and physiologic state. The biological effects of a given cytokine are, in turn, limited to target cells bearing the corresponding receptor.

Biochemical and somatic cell genetic studies have begun to resolve the mechanisms by which cytokines selectively modify receptor-bearing target cells. Studies of interferon α (IFN-α) and interferon γ (IFN-γ) have revealed essential components of the cytokine signaling pathway. Cells treated with IFN-γ rapidly activate an otherwise latent DNA binding protein (*2*). Concomitant with activation, the DNA binding protein, variously termed p91 or Stat1, becomes tyrosine-phosphorylated by way of a receptor-associated tyrosine kinase. Mutant cells lacking either Jak1 or Jak2 tyrosine kinase fail to respond to IFN-γ signaling. Shortly after phosphorylation of a single and specific tyrosine residue (Y701), p91 translocates from cytoplasm to nucleus where it activates an array of genes containing its specific binding site. The p91 protein has emerged as the founding member of a family of cytokine-activated transcription factors. Likewise, resolution of the role of Jak kinases in interferon signaling has uncovered a biological function for this class of tyrosine kinases. These observations provide a conceptual coupling between a specific cytokine and the battery of genes it induces in target cells.

Interleukin-4 (IL-4), like IFN-γ, rapidly alters the pattern of gene expression in cells bearing its cognate receptor. When exposed to IL-4, B lymphocytes activate the synthesis of sterile transcripts of the immunoglobulin locus and subsequently undergo class switching to the immunoglobulin E heavy chain isotype (*3*). IL-4 also activates genes encoding cell surface proteins including various immunoglobulin receptors and the MHC (major histocompatibility complex) class II antigen (*4*). IL-4 modulates the activity of DNA binding proteins in B lymphocytes (*5*). Three studies have indicated that IL-4 may regulate gene expression in a manner analogous to IFN-γ. A latent DNA binding protein is rapidly phosphorylated on tyrosine and translocated to the nucleus in receptor-bearing cells treated with IL-4 (*6*). In order to study the molecular mechanisms governing this process we have purified the IL-4–induced DNA binding protein and cloned its encoding gene.

Human monocytic Thp-1 cells were grown in suspension, treated briefly with IL-4, harvested, disrupted, and fractionated to separate nuclear and cytoplasmic proteins (*7*). Nuclear extracts from IL-4–treated cells, but not control cells, contained a DNA binding activity capable of specific interaction with a double-stranded, synthetic oligonucleotide corresponding to the IL-4 response element located upstream of the human FcγRI gene (*6*). This activity was purified by three chromatographic steps (*8*) and found to be specified by a polypeptide that migrated with a molecular size of 100 kD as indicated by SDS–polyacrylamide gel electrophoresis (Fig. 1). The 100-kD polypeptide reacted with an antibody that recognized phosphotyrosine, consistent with studies that implicated tyrosine phosphorylation as an essential step required for its activation (*6*).

The purified, 100-kD polypeptide was digested with lysine-C and resulting peptides were fractionated by capillary high-performance liquid chromatography. Amino acid sequences were obtained from six peptide fragments (*9*). Synthetic oligonucleotides designed from these sequences were used for

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