

Isolation and molecular characterization of the first genomic clone of a major peanut allergen, Ara h 2

Olga M. Viquez, PhD,^{a,b} Cathrine G. Summer, MS,^b and Hortense W. Dodo, PhD^a

Normal and Huntsville, Ala

Background: Peanuts have been identified as potent food allergens responsible for life-threatening IgE reactions among hypersensitive individuals. With the current increase of peanut allergies, there is an urgent need to molecularly characterize the genes encoding the target proteins and to understand the nature of their regulation.

Objectives: The objectives of this study were to isolate, sequence, and characterize at least one full-length genomic clone encoding the major peanut allergen Ara h 2.

Methods: A peanut genomic library, constructed in a Lambda Fix II vector, was screened with an 80-bp oligonucleotide probe constructed on the basis of the 5' end of a published Ara h 2 cDNA partial sequence. One putative positive lambda clone was isolated, digested with *Bam*HI to release its 16-kb insert, and confirmed by means of dot blot and Southern hybridization. The positive clone was subcloned in pBluescript SK+ vector, sequenced, and characterized.

Results: Sequence analysis revealed a full-length genomic clone with an open reading frame starting with an initiation codon (ATG) at position 1 and ending with a termination codon (TGA) at position 622. One putative polyadenylation signal (AATAAA) is identified at positions 951 in the 3' untranslated region, and 6 additional stop codons are located at positions 628, 769, 901, 946, 967, and 982 downstream from the start codon. In the 5' promoter region, a putative TATA box (TATTATTA) is located at position -72 upstream from the start codon. The deduced amino acid sequence has 207 residues and includes a putative signal peptide of 21 residues.

Conclusions: The results reveal for the first time information on the structure of a major peanut allergen, Ara h 2. Comparison of the cDNA and genomic sequences revealed the absence of an intron but the presence of 2 isoforms of Ara h 2 or different members of the same gene family. (*J Allergy Clin Immunol* 2001;107:713-7.)

Key words: Food allergy, peanut, allergens, genomic library, promoter, Ara h 2, genomic clone, major allergen

Abbreviations used

ORF: Open reading frame

SSC: Sodium chloride sodium citrate

SSPE: Sodium chloride sodium phosphate

Food allergies are a serious health problem of national importance. It is estimated that 3 million people in the United States have peanut or tree nut allergies.¹ Food allergies are increasing worldwide, and peanut is one of the most allergenic foods.

Peanut (*Arachis hypogaea* L.), a crop grown worldwide, is an inexpensive source of protein, minerals, vitamins, and unsaturated oil. However, multiple peanut proteins have been identified as allergens. Several allergenic proteins have been isolated, identified, characterized, and classified as minor or major allergens.²⁻⁸ The 2 major allergens are Ara h 1 and Ara h 2, to which more than 90% of peanut-hypersensitive individuals react.⁹ Ara h 2 is a glycoprotein of about 17 kd with at least 2 major bands and an isoelectric point of 5.2.¹⁰ A partial cDNA sequence has been determined. The amino acid sequence of Ara h 2 protein is composed of a high percentage of glutamic acid, aspartic acid, glycine, and arginine. Sequence analysis of Ara h 2 protein showed similarity to seed storage proteins of the conglutinin family, and the protein has at least 10 IgE epitopes.⁹

Ingestion of peanut has been implicated as the causative agent in cases of fatal or near-fatal anaphylaxis.¹¹⁻¹⁴ Allergic symptoms to peanut are rarely lost in hypersensitive individuals.¹²⁻¹⁶ Aside from avoiding exposure to peanut allergens, there is currently no effective treatment for individuals with peanut hypersensitivity. In addition, the peanut-hypersensitive population is at constant risk of accidental ingestion of peanut because of misleading product labeling, lack of labeling, contamination by hidden allergens, and the extensive use of peanut products in processed foods.^{12,14,17-19} An investigation of a wide variety of commercially grown peanuts showed no naturally occurring allergen-free peanut lines.²⁰ Modern molecular biologic tools have the potential to offer new transgenic allergen-free peanuts to the population with peanut allergy. Therefore an understanding of the molecular structure and regulatory features of the genes will provide needed information for protein silencing.

From ^athe Department of Food and Animal Sciences, Alabama A&M University, Normal; and ^bResearch Genetics Inc, Huntsville.

Supported by funds provided by the US Department of Agriculture-Capacity Building Program and the Alabama Agricultural and Experiment Station. Received for publication October 17, 2000; revised December 4, 2000; accepted for publication December 5, 2000.

Reprint requests: Olga Martha Viquez, PhD, Department of Food and Animal Sciences, Alabama A&M University, P.O. Box 1628, Normal, AL 35762.

Copyright © 2001 by Mosby, Inc

0091-6749/2001 \$35.00 + 0 1/87/113522

doi:10.1067/mai.2001.113522

The objectives of this study were (1) to isolate at least one full-length Ara h 2 clone from a peanut genomic library by using a synthetic oligonucleotide probe and (2) to sequence and analyze the structural and regulatory features of the gene encoding Ara h 2.

METHODS

Library screening

A peanut genomic library constructed from DNA extracted from seeds of *A hypogaea* (F78-1339) in a Lambda Fix II vector (Stratagene Inc, La Jolla, Calif) was screened with an 80-bp oligonucleotide probe. The probe sequence (5'-CTAGTAGCCCTCGCC-CTTTTCCTCCTCGCTGCCCCACGCATCTGCGAGGCAGCAGTGGGAACTCCAAGGAGACAGAAGATG-3') corresponds to nucleotides 11 to 91 of a published Ara h 2 cDNA sequence (GeneBank accession No. L77197).

Twenty picomoles of the probe was end-labeled with gamma ^{32}P , as described by Ausubel et al.²¹ Fresh *Escherichia Coli* VCS 257 (300 μL of 1×10^{10} cells/mL) was infected with 10 μL of the genomic library (1×10^3 plaque-forming units) for 30 minutes at 37°C in a water bath. Then 7 mL of top agarose (0.7% wt/vol) at 47°C was added, mixed, and spread onto a prewarmed (37°C) 150-mm 2 $\times$ LB agar plate.²² The plaques became visible after an overnight incubation at 37°C.

After plaque formation, the culture dishes were stored for 4 hours at 4°C, blotted on a piece of nylon membrane, denatured (NaOH, 0.5N), and neutralized (TRIS-HCl, 1 mol/L), according to the manufacturer's instructions (NEN Life Science Products, Inc, Boston, Mass), and the DNA was cross-linked at 12,000 μJ of UV energy for 45 seconds (UV Stratalinker 1800, Stratagene). Low-stringency prehybridization (at 42°C for 3 hours) and hybridization (at 42°C overnight) were performed in the same solution containing 50% (vol/vol) formamide, 10% (wt/vol) SDS, 20% (wt/vol) dextran sulfate, 1 $\times$ Denhardt's solution, and 10 $\mu\text{g}/\text{mL}$ salmon sperm DNA. During hybridization, the ^{32}P -labeled probe was added to the buffer. Membranes were washed 3 times with 2 $\times$ sodium chloride sodium citrate (SSC) followed by 2 $\times$ SSC and 0.1% (wt/vol) SDS for 15 minutes at room temperature, air-dried, exposed to Kodak XAR-5-X ray film, and developed after 7 days at -80°C. Positive clones were matched with plaques on the Petri dishes, lifted, and stored at 4°C in 1 mL of SM media containing a few drops of chloroform to prevent bacterial contamination.²² To confirm true-positive clones, a second screening was performed, as described above.

Purification of putative positive clones

Selected putative positive clones were amplified, as described by Sambrook et al.²² Lysate stocks of recombinant bacteriophage were prepared by infection of *E coli* VCS 257 with each putative positive clone. The cultures were grown for 6 to 8 hours at 37°C and 300 rpm. Purification of lambda DNA was done with a Lambda kit (Qiagen Inc, Valencia, Calif), and the DNA was quantified by using a fluorometer.²³

Dot blot analysis

Positive clones were confirmed by means of dot blot analysis with a Bio-Dot SF Microfiltration apparatus (Bio-Rad Laboratories, Inc, Hercules, Calif) and the Southern hybridization protocol.²⁴ One microgram of each purified DNA was blotted and transferred by means of capillary action to a nylon membrane. DNA was cross-linked to the membranes at 12,000 μJ of UV energy for 45 seconds. The membrane was prehybridized at 50°C for at least 4 hours in 6 $\times$ sodium chloride sodium phosphate (SSPE), 5 $\times$ Denhardt's solution, 0.05% (wt/vol) NaPyrPO₄, 0.5% (wt/vol) SDS, and 100 $\mu\text{g}/\text{mL}$ salmon sperm DNA and hybridized at 50°C overnight in 6 $\times$ SSPE,

1 $\times$ Denhardt's solution, 0.05% NaPyrPO₄, and 0.5% SDS with the same ^{32}P end-labeled probe used to screen the library. Stringent washes were performed at 50°C for 15 minutes each in 6 $\times$ SSPE with 0.1% (wt/vol) SDS and 2 $\times$ SSPE with 0.1% (wt/vol) SDS. After air-drying, the membrane was exposed to Kodak X-Omar AR film at -80°C for 2 days and autoradiographed.

Subcloning

The selected positive lambda clone for Ara h 2 was subcloned into a pBluescript II SK(+/-) phagemid vector (Stratagene) to facilitate sequencing.

Restriction enzyme digestion

Restriction enzyme digestion with *Bam*HI was performed at 37°C. Fragments were separated by means of electrophoresis on a 0.7% (wt/vol) agarose gel. Five fragments (5.5, 6.5, 9, 12, and 16 kb) were obtained. Each fragment was cut from the agarose gel, filtered through a Millipore Ultrafree-DA filter (Millipore Corp, Bedford, Mass), and precipitated in 100% ethanol. The digested pBluescript II vector was dephosphorylated with calf intestinal alkaline phosphatase before ligation with the DNA fragments, purified with an equal volume of phenol-chloroform, precipitated in ethanol, and resuspended in one volume of buffer (5 mmol/L TRIS [pH 7.5] and 0.1 mmol/L EDTA) to a final concentration of approximately 0.1 $\mu\text{g}/\mu\text{L}$.

Ligation

A 2:1 and 3:1 ratio of insert to vector DNA was selected. The ligation reaction was performed at 4°C overnight and then at room temperature for 3 hours. About 20 μL of ultracompetent bacteria cells (*E coli* DH10B; GENEHOGS, Research Genetics, Huntsville, Ala) were mixed with 1 μL of ligation mixture, electroporated, and resuspended in 1 mL of 37°C sterile SOC medium, as described in the GENEHOGS protocol (Research Genetics). Electroporation was performed by using a Bio-Rad Gene Pulser electroporator (Bio-Rad Laboratories, Richmond, Calif) with the following settings for a 1-mm gap electroporation cuvette (BTX Genetronics, Inc, San Diego, Calif): the field strength at 17 kV/cm, the resistor at 200 Ω , and the capacitor at 25 μF . Positive colonies were chosen on the basis of blue-white color selection. White positive colonies containing a plasmid with an insert were picked²⁵ from each plate and placed onto 6 mL of LB media supplemented with ampicillin (100 $\mu\text{g}/\text{mL}$) and incubated at 37°C for 16 hours at 300 rpm. Plasmid DNA was purified by using the Qiagen Plasmid Purification kit, digested with *Bam*HI, and separated on 0.7% agarose gel to confirm the presence of a plasmid containing an Ara h 2 insert.

Southern hybridization

Digested DNA fragments were transferred onto a nylon membrane by using an alkaline transfer protocol, according to the manufacturers' instructions (Pall, NEN Life Science Products). The DNA was cross-linked on the membrane as previously described and prehybridized at 65°C for 3 hours in HyperHyb buffer (Research Genetics, Inc). The probe was end-labeled with ^{32}P as described in the Fermentas kit (Fermentas Inc, Hanover, Md), added to the hybridization solution, and incubated at 65°C for 3 hours in HyperHyb buffer. The membrane was washed 3 times at 65°C for 15 minutes each in 0.1 $\times$ SSC and 0.1% SDS, rinsed once at room temperature in 1 $\times$ SSC, exposed to x-ray film (Kodak, Biomax MS) at -80°C for 3 hours, and autoradiographed.

Sequencing

Purified positive pBluescript DNA (0.2 $\mu\text{g}/\mu\text{L}$) were sequenced with ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction kit by using AmpliTaq DNA Polymerase FS at Research Genetics, Inc, and at the University of Alabama in Birmingham by using T3 and T7 sequencing primers.

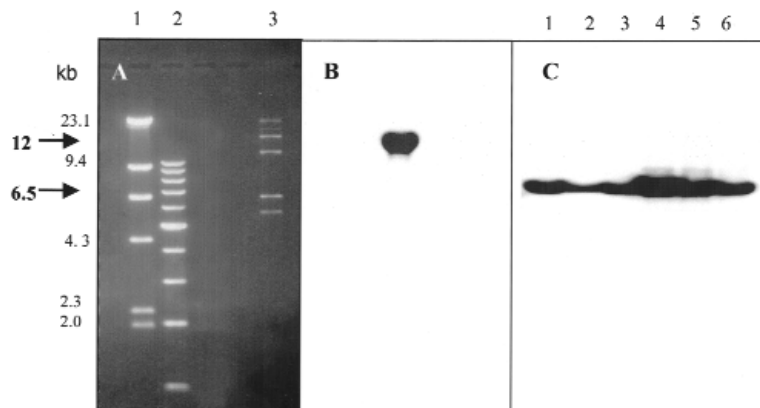


FIG 1. **A**, Lane 1, Lambda DNA/*Hind* III markers; lane 2, 1-kb DNA step ladder; lane 3, *Bam*HI digestion pattern of a positive 50-kb Lambda genomic clone for the gene encoding Ara h 2. **B**, Southern hybridization of a 12-kb *Bam*HI fragment subcloned into pBluescript II SK(+/-) by using the 80-bp ^{32} P-labeled probe designed on the basis of Ara h 2 cDNA. **C**, Southern hybridization of a 6.5-kb *Bam*HI fragment subcloned into pBluescript II SK(+/-) by using the 62-bp ^{32} P-labeled probe designed on the basis of Ara h 2 cDNA (clones 1-6 represent 6 positive pBluescript clones).

Sequence analysis

Sequence analysis, comparison, and homology searches were performed by using the BLAST²⁶ and BLAST 2 sequences tools.²⁷ Determination of leader sequence was done as described by Grierson and Covey.²⁸

RESULTS

Library screening

Approximately one million clones of a peanut genomic library were screened with a ^{32} P-labeled 80-bp synthetic oligonucleotide probe. In the first screening, 24 putative positive clones were obtained, and 5 were obtained in the second screening. The DNA of each clone was isolated, purified, and dot blotted onto a nylon membrane. Only one of those clones hybridized to the probe.

Subcloning of a 12-kb fragment into a pBluescript II SK+ plasmid vector

The selected positive lambda clone was approximately 50 kb, with an insert fragment of about 16 kb. The clone was digested with *Bam*HI to release the insert and electrophoresed on a 0.7% agarose gel. Five fragments, ranging in size from 5.5, 6.5, 9, 12, and 16 kb, were obtained. After Southern hybridization, only the 12-kb fragment hybridized to the ^{32} P-labeled 80-bp probe, and it was gel purified and subcloned into a pBluescript II SK+ plasmid vector (Fig 1). Sequence analysis revealed that the selected 12-kb DNA fragment was truncated at a *Bam*HI restriction site located about 212 nucleotides within the gene.

Subcloning of a 6.5-kb fragment into a pBluescript II SK+ plasmid vector

A 62-bp oligonucleotide (5'-GTGCATGTGCGAG-GCATTGCAACAGATCATGGAGAACCAGAGC-GATAGTTGCAGGGGAGGC-3') was designed from nucleotides 301 to 362 of the cDNA sequence down-

stream from the *Bam*HI restriction site to capture the remaining DNA fragment of the gene encoding Ara h 2. Of the 5 fragments obtained after digestion of the 50-kb lambda clone with *Bam*HI, only the 6.5-kb fragment hybridized to this probe. This fragment was subcloned into pBluescript II SK+ plasmid vector, confirmed by Southern hybridization, and sequenced (Fig 1).

Sequence analysis

Both the sense and antisense strands of the 2 fragments subcloned in pBluescript SK+ were sequenced. Analysis of the sequence revealed a full-length gene encoding Ara h 2. The open reading frame (ORF) starts with an initiation codon (ATG) at position 1 and ends with a termination codon (TGA) at position 622. The predicted encoded protein is 207 amino acids long and includes a putative transit peptide of 21 residues. One putative polyadenylation signal (AATAAA) is identified at position 951. Six additional putative stop codons are observed downstream of the first termination codon at positions 628 (TGA), 769 (TAA), 901 (TAA), 946 (TGA), 967 (TGA), and 982 (TGA). In the promoter region, 5' upstream of the start codon, a putative TATA box (TATTATTA) is present at position -72. Comparison of the published cDNA and genomic sequences revealed the absence of an intron.

DISCUSSION

Our results reveal, for the first time, information on the structure of the genomic clone and regulatory sequences of a peanut allergen gene (GeneBank accession No. AY007229).

The location of the initiation codon ATG of Ara h 2 is revealed for the first time. Until now, only partial cDNA sequences have been published.⁹ The ORF of the genomic clone of Ara h 2 is 621 nucleotides long, whereas its cDNA (GeneBank accession No. L77197) is 492

```

                                tccttacgcgaaatacggg
-91  cagacatggcctgccccggttattattattttttgacacagaccaac
-46  tggtaatggtagcgaccggcgctcagctggaattcgcggccgcca
   1  atggccaagctcaccatactagtagccctcgcccttttcctcctc
      M A K L T I L V A L A L F L L
  46  gctgcccacgcatctgcgaggcagcagtggaactccaaggagac
      A A H A S A R Q Q W E L Q G D
  91  agaagatgccagagccagctcgagaggcgaaacctgaggccctgc
      R R C Q S Q L E R A N L R P C
 136  gagcaacatctcatgcagaagatccaacgtgacgaggattcatat
      E Q H L M Q K I Q R D E D S Y
 181  gaacgggacccgtacagccctagtcaggatccgtacagccctagt
      E R D P Y S P S Q D P Y S P S
 226  ccatatgatcggagaggcgctggatcctctcagcaccaagagagg
      P Y D R R G A G S S Q H Q E R
 271  tgttgcaatgagctgaacgagtttgagaacaaccaaaggtgcatg
      C C N E L N E F E N N Q R C M
 316  tgcgaggcattgcaacagatcatggagaaccagagcgataggttg
      C E A L Q Q I M E N Q S D R L
 361  caggggaggcaacaggagcaacagttcaagaggagctcaggaac
      Q G R Q Q E Q Q F K R E L R N
 406  ttgcctcaacagtgcggccttagggcaccacagcgttgcgacttg
      L P Q Q C G L R A P Q R C D L
 451  gacgtcgaaagtggcggcaggcgccgcgaattccgcccatactg
      D V E S G G R R P R I P P I L
 496  acgggctccaggagtcgtcgccaccaatcccatatggaaaccgt
      T G S R S R R H Q S P Y G N R
 541  cgatattcagccatgtgccttcttccgcgtgcagcagatggcgat
      R Y S A M C L L P R A A D G D
 586  ggctggtttccatcagttgctgttgactgtagcggctgatgttga
      G W F P S V A V D C S G Stop
 631  actggaagtcgccgcgccactgggtgtgggccataattcaattcgc
 676  gcgtcccgcagcgcagaccgttttcgctcgggaagacgtacgggg
 721  tatacatgtctgacaatggcagatcccagcgggtcaaacaggcgg
 766  cagtaaggcggtcgggatagttttcttgcggccctaattccgagcc
 811  agtttaccgcgtctgtctacctgcgccagctggcagttcaagccaa
 856  tccgcgcggatgcggtgtatcgctcgccacttcaacatcaacgg
 901  taatcgccatttgaccactaccatcaatccggtaggttttcggc
 946  tgataaataaaggttttccctgatgtgtgccacgcgtgagcggtc
 991  gtaatcagcaccgcacatcaacaagtgtattttgccgtgcaactgcaa
1036  caacgctggttcgggctg

```

FIG 2. Nucleotide and deduced amino acid sequences of the gene encoding the peanut allergen Ara h 2. From top to bottom, a putative TATA box, the ATG initiation codon, the first stop codon (TGA), and the putative polyadenylation signal are in bold. The 21 putative amino acid signal peptide is underlined. Six additional stop codons are underlined. The deduced amino acid sequence is below the nucleotide sequence.

nucleotides long. A comparison of the 2 sequences revealed that the cDNA sequence is 8 nucleotides short at the 5' region and does not include a start codon. In addition, the 2 sequences have complete identity from nucleotides 9 to 470 of the genomic clone. However, from nucleotide 471, they diverge with no homology downstream from this region at the nucleotide, as well as the amino acid levels. This confirmed the presence of isoforms for Ara h 2 in the peanut genome, the presence of different members of the same gene family, or both. It should also be taken into consideration that the cDNA and genomic clones derive from 2 different peanut varieties. The mRNA used to originate the cDNA was

extracted from a Florunner, whereas the genomic clone was isolated from a peanut library from seed DNA *A hypogaea* (F78-1339). Thus sequence divergence could be due to varietal differences.

The termination codon is TGA at position 622. Not only is the termination codon use different between the genomic (TGA) and the cDNA (TAA) clone, but the latter also ends 152 bp, or 51 amino acids, earlier than the genomic clone. Six additional stop codons are present in the 3' untranslated region at positions 628 (TGA), 769 (TAA), 901 (TAA), 946 (TGA), 967 (TGA), and 982 (TGA). It is known that some genes have several termination codons²⁸; however, it is unclear which one is preferentially used. A

gene usually undergoes posttranscriptional and posttranslational modifications, which could explain some of the differences between the genomic and cDNA sequences.

A putative polyadenylation signal, AATAAA, is located at position 951 in the 3' untranslated region of the gene. This signal is identical to the consensus sequence for plants. Polyadenylation signals play a key role in the stability and translation of the genetic message and direct the termination of transcription by RNA polymerase II.^{29,30}

The deduced polypeptide encoded by the ORF has 207 amino acid residues and includes a putative signal peptide of 21 amino acid residues (Fig 2).³¹ A signal peptide plays a role in the translocation of a protein from the cytosol to the target organelle within the cell.³² It is typically composed of hydrophobic amino acids, such as tryptophan, phenylalanine, valine, leucine, and isoleucine, which have affinity for membranes of organelles.³²

The promoter region of a gene is typically composed of regulatory sequences at the 5' end of the transcription unit and directs the initiation of transcription by RNA polymerase II. In the proximal region of the promoter, a putative TATA box (TATTATTA) is present at position -72 with respect to the initiation codon. The consensus signal for plant TATA boxes is TATA^T/_AA₁₋₃.²⁸ This is the most conserved sequence for RNA polymerase II-mediated transcription and is important for positioning the start of transcription.^{32,33}

Promoter-mediated control is one mechanism by which peanut allergens can be downregulated. Therefore an understanding of the structure of the gene, the conserved regions, consensus sequences, and the flow of the genetic information will provide good insight in designing a strategy to engineer an allergen-free peanut plant.

We thank Dr Albert G. Abbott (Biological Sciences Department, Clemson University, Clemson, SC) for providing the peanut genomic library and Dr Maria Ragland (Research Genetics, Inc, Huntsville, Ala) for her scientific advice.

REFERENCES

1. Sicherer SH, Muñoz-Furlong A, Burks AW, Sampson HA. Prevalence of peanut and tree nut allergy in the US determined by a random digit dial telephone survey. *J Allergy Clin Immunol* 1999;103:559-62.
2. Burks AW, Williams LW, Helm RM, Connaughton C, Cockrell G, O'Brien T. Identification of a major peanut allergen, *Ara h* 1, in patients with atopic dermatitis and positive peanut challenges. *J Allergy Clin Immunol* 1991;88:172-9.
3. Burks AW, Cockrell G, Connaughton C, Helm RM. Allergens, IgE, mediators, inflammatory mechanisms: epitope specificity and immunoaffinity purification of the major peanut allergen, *Ara h* 1. *J Allergy Clin Immunol* 1994;93:743-50.
4. Burks AW, Sampson HA, Bannon GA. Peanut allergens. *Allergy* 1998;53:725-30.
5. Kleber-Janke T, Cramer R, Appenzeller U, Becker WM, Schlaak M. Selective cloning of peanut allergens, including profilin and 2S albumins, by phage display technology. *Int Arch Allergy Immunol* 1999;119:265-74.
6. Rabjohn P, Helm EM, Stanley J, West CM, Sampson H, Burks AW, et al. Molecular cloning and epitope analysis of the peanut allergen, *Ara h* 3. *J Clin Invest* 1999;103:535-42.
7. Sachs MI, Jones RT, Yunginger JW. Isolation and partial characterization of a major peanut allergen. *J Allergy Clin Immunol* 1981;67:27-34.
8. Bannon GA, Li XF, Rabjohn P, et al. *Ara h* 3, a peanut allergen identified by using peanut sensitive patient sera absorbed with soy proteins. *J Allergy Clin Immunol* 1992;90:962-9.
9. Stanley JS, King N, Burks AW, Huang SK, Sampson H, Cockrell G, et al. Identification and mutational analysis of the immunodominant IgE binding epitopes of the peanut allergen *Ara h* 2. *Arch Biochem Biophys* 1997;342:244-53.
10. Burks AW, Williams LW, Connaughton C, Cockrell G, O'Brien TJ, Helm RM. Identification of a second major peanut allergen, *Ara h* II, with use of the sera of patients with atopic dermatitis and positive peanut challenge. *J Allergy Clin Immunol* 1992;90:962-9.
11. Sampson HA. Food allergy. I. Immunopathogenesis and clinical disorders. *J Allergy Clin Immunol* 1999;103:717-28.
12. Sicherer SH, Furlong T, DeSimone J, Sampson HA. Self-reported allergic reactions to peanut on commercial airliners. *J Allergy Clin Immunol* 1999;104:186-9.
13. Oppenheimer JJ, Nelson HS, Bock A, Christensen F, Leung DYM. Treatment of peanut allergy with rush immunotherapy. *J Allergy Clin Immunol* 1992;90:256-62.
14. Yunginger JW, Sweeney KG, Sturmer WQ, Giannandrea LA, Teigland JD, Bray M, et al. Fatal food-induced anaphylaxis. *JAMA* 1988;260:1450-2.
15. O'Neil CE, Lehrer SB. Seafood allergy and allergen: a review. *Food Technol* 1995;49:103-14.
16. Taylor SL. Chemistry and detection of food allergens. *Food Technol* 1992;46:46-152.
17. Sampson HA. Peanut anaphylaxis. *J Allergy Clin Immunol* 1990;86:1-3.
18. Steinman HA. "Hidden" allergens in foods. *J Allergy Clin Immunol* 1996;98:241-50.
19. Nordlee JA, Taylor SL, Jones RT, Yunginger JW. Allergenicity of various peanut products as determined by RAST inhibition. *J Allergy Clin Immunol* 1981;68:376-82.
20. Dodo HW, Marsic D, Callender M, Cebert E, Viquez OM. Screening peanut germplasm for allergen content. International Food and Nutrition Conference 2000. Tuskegee, AL. Book of Abstracts: 15.
21. Ausubel F, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, et al. Short protocols in molecular biology. 3rd ed. New York: John Wiley; 1995.
22. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: a laboratory manual. 2nd ed. New York: Cold Spring Harbor Laboratory Press; 1989.
23. TKO 100DNA Mini Fluorometer Instruction Manual. Hoefer Scientific Instruments; 1991.
24. Southern EM. Detection of specific sequences among DNA fragments by gel electrophoresis. *J Mol Biol* 1975;98:503-17.
25. Instruction manual: pBluescriptIIExo/Mug DNA Sequencing System. La Jolla (CA): Stratagene Incorporation; 1999.
26. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 1997;25:3389-402.
27. Tatusova TA, Madden TL. Blast 2 sequences, a new tool for comparing protein and nucleotide sequences. *FEMS Microbiol Lett* 1999;174:247-50.
28. Grierson D, Covey SN. Plant molecular biology. 2nd ed. New York: Chapman and Hall Publishers; 1988.
29. Birse C, Proudfoot N. A functional polyadenylation signal and a downstream transcription 'pause' element are required for efficient pol II transcription termination in fission yeast. Available at: <http://genome-www.stanford.edu/Saccharomyces/yeast96/tf2021.html>. Accessed in 1996.
30. Greger IH, Proudfoot NJ. Poly(A) signal control both transcriptional termination and initiation between the tandem GAL10 and GAL7 genes of *Saccharomyces cerevisiae*. *EMBO J* 1998;17:4771-9.
31. Nielsen H, Engelbrecht J, Brunak S, von Heijne G. Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites. *Protein Eng* 1997;10:1-6.
32. Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson JD. Molecular biology of the cell. 3rd ed. New York: Garland Publishing, Inc; 1994.
33. Ellison K, Messing J. The molecular architecture of plant genes and their regulation. *Biotechnology* 1983;12:115-39.