Food Allergies –
A Challenge for Current and Emerging Proteins

Steve L. Taylor, Ph.D.
Food Allergy Research & Resource Program
University of Nebraska

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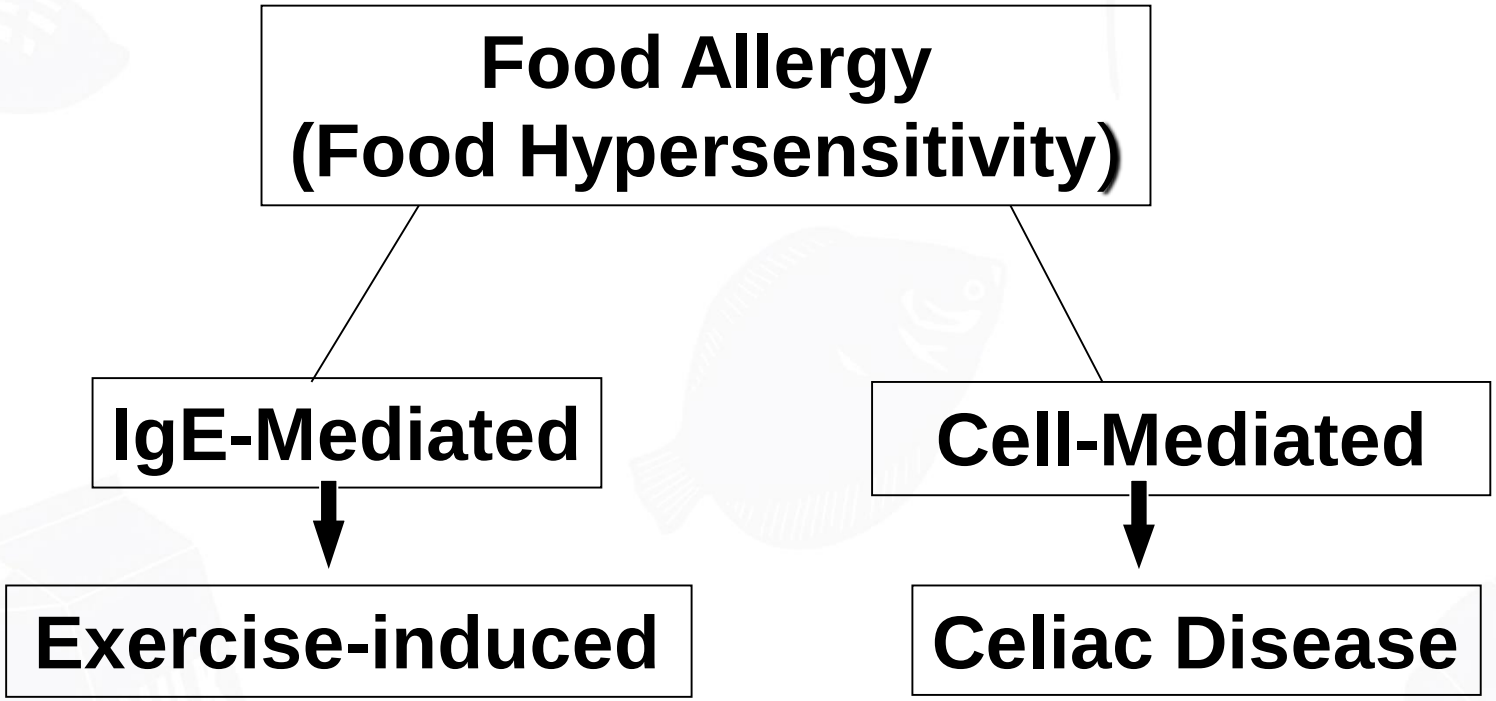
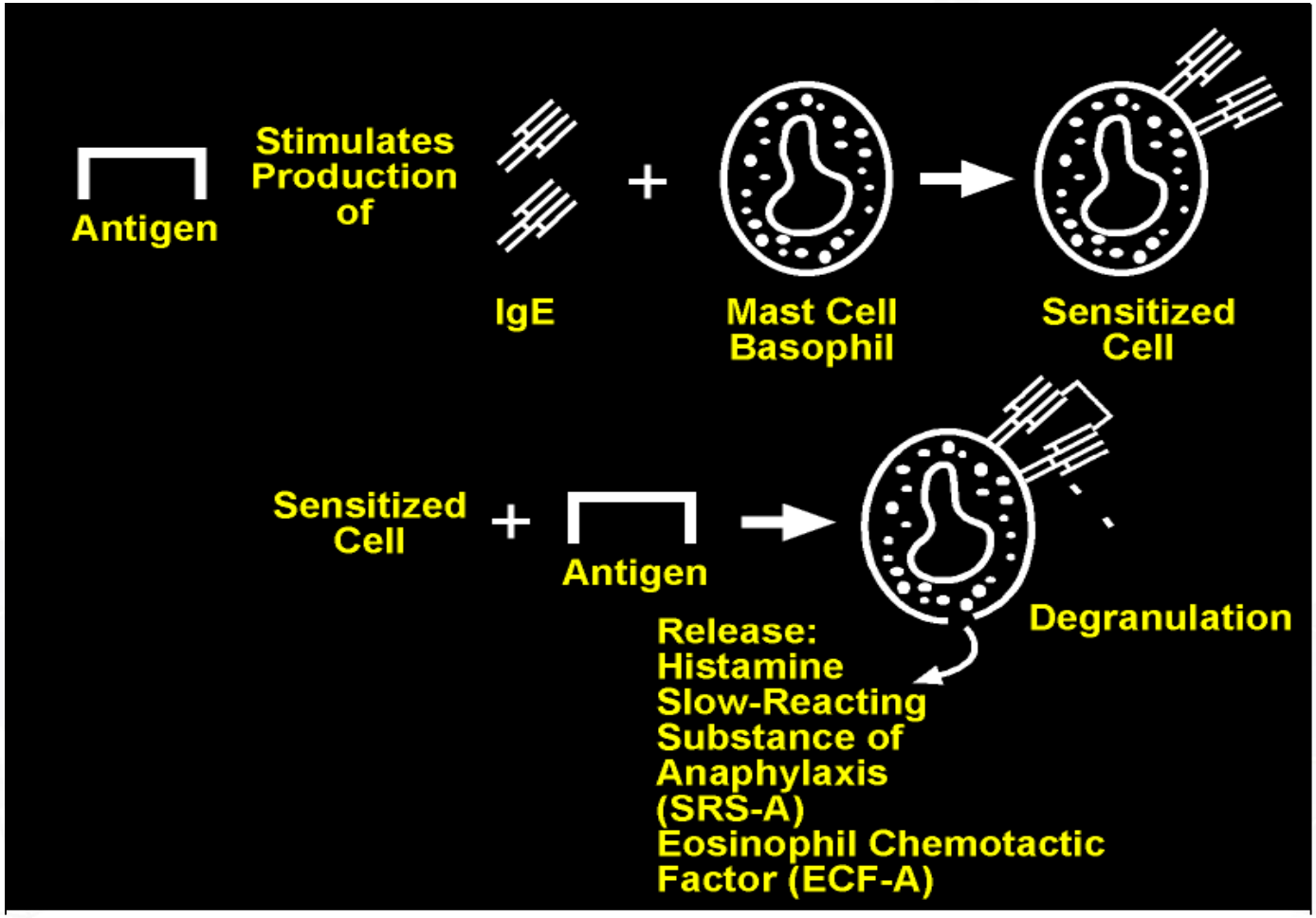
Food Allergies

- Food allergens are **proteins**
- Food allergies increasing in prevalence in U.S. and other developed countries; 15 million Americans have food allergy and 3 million have celiac disease
- Symptoms range from mild and transient to severe and life-threatening
- Potency is another key concern as exposure to trace amounts can provoke allergic reactions
- Abnormal responses of immune system


Food Allergy
(Food Hypersensitivity)
IgE-Mediated
Cell-Mediated
Exercise-induced
Celiac Disease

Mechanisms of Mediator Release



The Big 8
Most Common Causes of Food Allergy
(IgE-Mediated)

- Cows' milk
- Egg
- Crustacea
- Fish
- Peanut
- Soybean
- Tree nuts
- Wheat

Responsible for 90% of all food allergies globally

Allergenic Foods on Common Allergen Lists in Other Countries

• Sesame seed	Canada, EU, Australia/ New Zealand
• Mustard	EU, Canada
• Celery	EU
• Buckwheat	Japan, Korea
• Molluscan Shellfish	Canada, EU
• Lupine	EU

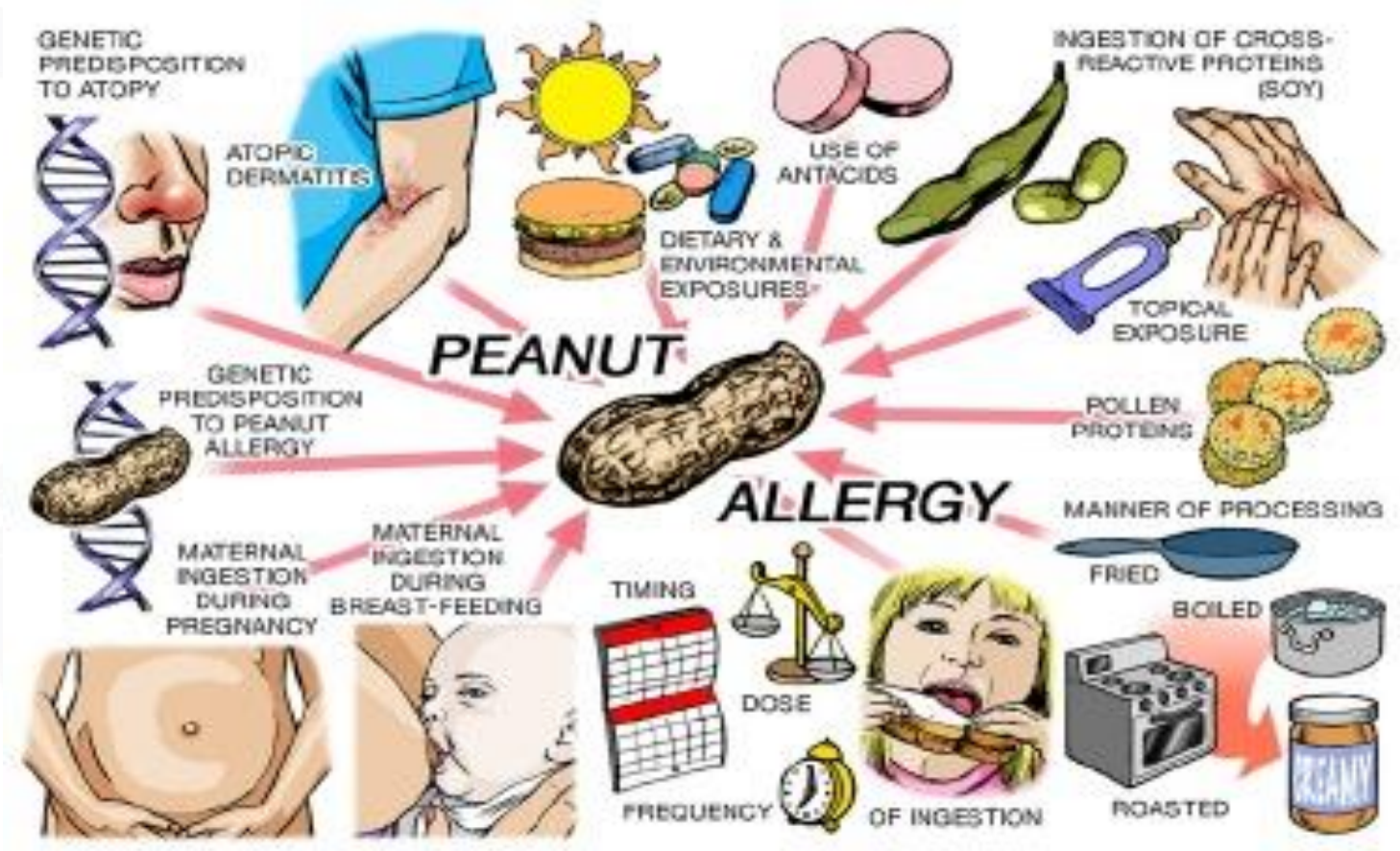
Why Do Certain Protein Sources Make These Allergen Lists?

- Clinically significant allergens – true of the Big 8 but maybe not for all (celery, mustard in EU)
- Prevalence of allergy – but good data are lacking
- Severity – yes but hard to track
- Potency – should be important
- Could my favorite new protein source end up on one of these lists? The lupine story

Characteristics of Commonly Allergenic Foods

- Frequently consumed
- Consumed in relatively large amounts
- Consumed in early life stages (with exceptions)
- Excellent sources of protein
- Allergenic sensitizing capacity of individual proteins in the food matrix

Factors Involved in Development of IgE-Mediated Food Allergy



Sicherer and Sampson. J Allergy Clin Immunol. 2007; 120:491-503.

Could My Favorite Novel Food Protein Source Become Allergenic?

- Inevitable; reports of allergic reactions should not be a surprise
- But perhaps not commonly; prevalence will be predictable to some extent
- Not all adverse reactions will be allergic reactions
- Should not be a deterrent to development of new protein sources
- Clear labeling is the key

Could Novel Food Sources of Protein Become Allergenic?

- Soybean – from obscurity to the Big 8
- Quorn – all reactions may not be allergies
- Lupine – an unexpected surprise
- Pea protein – lupine revisited??
- Canola protein isolate - predictable
- Insects as foods
- Wheat protein isolate – modifications can make a difference

Could Novel Food Sources of Protein Become Allergenic?

- Soybeans
- Quorn
- Lupine
- Pea protein
- Canola protein isolate
- Insects as foods
- Wheat protein isolate

Soybeans

- First domesticated in China in ~ 1100 BC
- Introduced in U.S. in 1765
- But first widely grown in U.S. in 1940s due to WWII impacts on production in China
- First soy foods for humans were Asian origin: tofu, soy sauce, etc.
- 1930 – soy infant formula developed but more widely used in 1950s
- 1959 – soy protein isolates first introduced

Soybean Allergy

- Prevalence increased from 1950s because milk-allergic infants were fed soy formula and some developed allergy to soy
- Soy formula – primary (sole) source of nutrition; soy primary source of protein
- Higher intake of soy protein from many sources in U.S. diet beginning in 1960s
- Most children outgrow soy allergy but still Big 8
- Not a very potent allergenic food

Should Product Developers Still Worry About Soybean Allergy?

- Company introduced muscle-building beverage with very high levels of soy and milk protein
- Received multiple consumer complaints including 6 reports of severe reactions in peanut-allergic subjects
- No peanut residues in beverage
- Using sera from 4 of these subjects, FARRP determined that all were highly allergic to peanut and slightly allergic to soy
- Tentative conclusion: the beverage had enough soy protein to exceed threshold dose of these consumers

Could Novel Food Sources of Protein Become Allergenic?

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Quorn

- Mycoprotein or mycelial mass with high protein and fiber content
- Product release as Quorn in U.K. in 1985; GRAS approval in U.S. in 2001
- Enjoys reasonable popularity in U.K.
- Adverse reactions reported by minority of consumers; most only with GI symptoms; likely due to individual problems in handling the high fiber content
- Handful of allergic reactions reported since 1985

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Lupine

- A legume with at least 4 edible species but historically used mostly as cattle feed
- In EU, lupine flour and other lupine protein ingredients introduced as replacements for soy flour, isolate and concentrate
- In Australia, lupine flour and other lupine protein ingredients introduced as novel foods
- In EU principally, allergic cross-reactions were noted in some peanut-allergic individuals; not so common in Australia
- Lupine now on priority allergen lists in EU and Australia

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Pea Protein

- Peas are a traditional legume crop eaten both as garden peas and as dry peas
- Pea protein ingredients developed recently and have surged in popularity in past several years
- Pea allergy from garden pea is rather rarely encountered but protein exposure is low
- Pea protein ingredients will serve to increase pea protein exposure
- Does pea protein cross-react with peanut?
- Analytical challenges

Could Novel Food Sources of Protein Become Allergenic?

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Canola Protein Isolate

- Canola is widely used as oil source but meal was not historically fed to humans due to presence of anti-nutrients
- Several companies have made canola protein isolate with no detectable anti-nutrients and usefuil technological properties
- Canola, rapeseed, and mustard are very closely related
- Mustard-allergic consumers are very likely to react to canola protein isolate

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Insects as Food

- Insects (meal worm, silk worm, cricket, etc.) can serve as excellent sources of protein and other nutrients
- Yuck factor overcome by use of forms such as cricket flour
- Insects are invertebrates so concern about possible allergic cross reactions with other invertebrates, namely crustacean and molluscan shellfish
- Tropomyosin is a major allergen in shrimp but also exists in insects; cross-reactivity??

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Wheat Protein Isolate

- During the Atkins Diet/low-carb period, many new, functional sources of proteins were developed
- Several variations of wheat protein isolates were introduced with differing technological functionality
- One wheat protein isolate introduced especially in EU as a coffee creamer
- Allergic reaction complaints surfaced, curiously among individuals who could eat wheat flour
- Most allergic reactions associated with one particular modified from of WPI; removed from market

Can We Predict the Allergenic Potential of Novel Food Sources of Protein?

- Is the food already known to be allergenic when consumed in more limited quantities? e.g. pea
- Is the food commonly allergenic in another country where it is more commonly consumed? e.g. buckwheat
- Is the food botanically related to known allergenic foods? e.g. soy, lupine, pea, insects
- Does the food contain a potentially cross-reactive protein? e.g. lupine

Prediction Examples

- Cottonseed protein – severe allergy cases reported from limited consumption
- Canola protein – very closely related to mustard
- Buckwheat – commonly allergenic in Japan and Korea
- Lupine – a legume (but there are 100s)
- Pea – a legume (but there are 100s)
- Insects – potentially cross-reactive protein with crustacean shellfish

Recommended Actions

- Search global clinical allergy literature for evidence of allergic reactions to the novel food
- Identify and assess allergenicity of botanically related species
- Determine abundance and amino acid sequence homology of individual proteins that are from the same class as major allergens from closely related sources
- Test for cross-reactivity and digestive stability

Predicting the Allergenicity of Individual Food Proteins

- Humans consume 1000s of plant and animal proteins in the diet on a daily basis; all dietary proteins are foreign proteins to the human immune system
- Only a small number of proteins from plant and animal origin cause an IgE-mediated immune response in only a small number of humans
- Oral tolerance is the homeostatic condition for dietary proteins
- Why are some proteins allergenic while others are not?

Why Are Some Food Proteins More Allergenic?

- Comparative abundance
- Usually major proteins of the food
- Heat-resistant
- Resistant to digestion/proteolysis
- Resistant to extremes in pH
- Foods can have 1 or many allergens in them

Characteristics of Food Allergens

- Abundance:
  - Most of the major food allergens are present a levels >1% of the total protein in the food
  - Immune system more likely to encounter abundant proteins
  - Seed storage proteins are especially abundant
- IgE-binding epitopes:
  - Most food allergens have multiple, linear epitopes and often these are arranged as conformational epitopes

Characteristics of Food Allergens

- Resistance to denaturation and digestion:
  - Highly stable proteins have the opportunity to interact with immune system for longer periods
- Allergen structure:
  - Secondary and tertiary structure may contribute to stability
    - Many food allergens contain disulfide bonds that stabilize the protein structure
    - Other allergenic food proteins form aggregates that enhance stability

Classification of Food Allergens

- Food allergens are commonly classified into families by their shared amino acid sequences and conserved 3-D structures
- Bioinformatics analysis indicates that the majority of food allergens from plant sources fall in 4 families
- Food allergens from animal sources fall within 3 main families

Classification of Food Allergens

- 4 main families of plant-based food allergens:
  - Prolamin superfamily
  - Cupin superfamily
  - Bet v 1 family
  - Profilins
- 3 main families of animal-based food allergens
  - Tropomyosins
  - EF-hand proteins
  - Caseins

Prolamin Superfamily

- Characterized on the basis of the presence of a conserved 8 cysteine amino acid residue pattern $CX_nCX_nCCX_nCXCX_nCX_nC$
  - Stabilizes protein structure which contributes to overall allergenicity of proteins in this class (highly resistant to heating, proteolysis and digestion)
- Share a common 3-D structure consisting of bundles of 4 α-helices stabilized by disulfide bonds
- Includes cereal prolamins, 2S albumins, non-specific lipid transfer proteins, and α-amylase and trypsin inhibitor protein families

Prolamin Superfamily

- Gliadin
  - Major seed storage protein from wheat
  - Major component of gluten
  - Involved in gluten sensitivity (celiac disease)
  - Related proteins in barley, rye, triticale, spelt

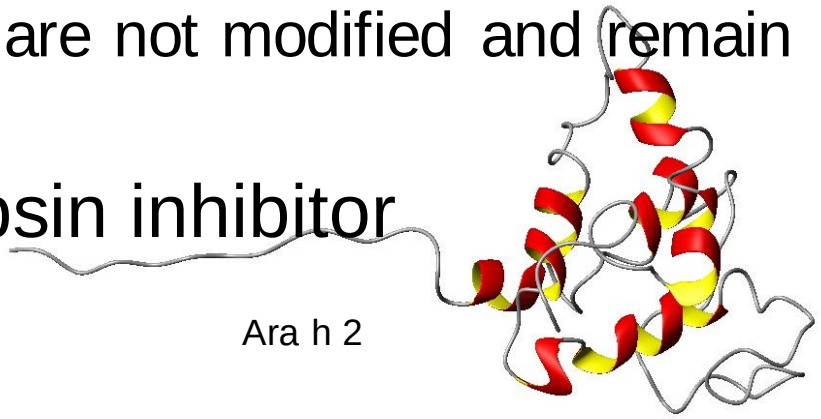
- nsLTPs
  - Facilitate transfer of phospholipids; plant defense against fungal and bacterial infections
  - Mal d 3 (apple), Pru ar 3 (cherry), Pru p 3 (peach), Jug r 3 (walnut), Cor a 8 (hazelnut), Aspa o 1 (asparagus), Lac s 1 (lettuce), Bra o 1 (cabbage)

Prolamin Superfamily

- α-amylase/trypsin inhibitors
  - Provide protection against degradative proteases produced by insect pests and pathogens
    - Rag 1, 2, 5 (rice)
- 2S albumins
  - Seed storage proteins
    - Jug r 1 (walnut), Ber e 1 (Brazil nut), Ana o 3 (cashew), Sin a 1 (yellow mustard), Bra j 1 (black mustard), Ara h 2, 6, 7 (peanut), Ses i 2 (sesame), SFA-8 (sunflower)

Prolamin Superfamily

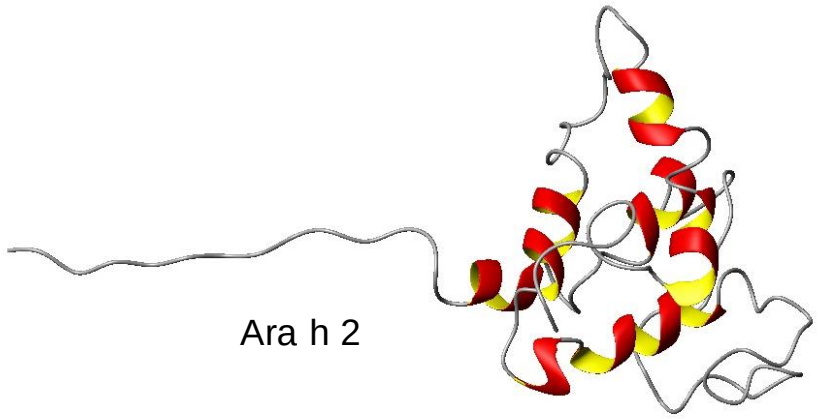
- 2S Albumins:
  - Seed storage proteins of dicotyledonous plants
  - Synthesized as single chains of 10-15 kDa
    - Many post-translationally processed into small and large subunits joined by disulfide bonds
    - Peanut and sunflower are not modified and remain as a single unit
  - Ara h 2 has some trypsin inhibitor properties



Ara h 2

Prolamin Superfamily

- 2S Albumins:
  - Major allergens in peanut, mustard, sesame seed, walnut, cashew, sunflower seed
  - Involved in provocation of severe allergic reactions

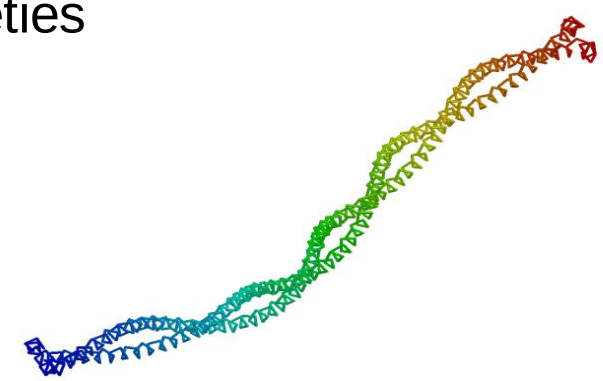


Novel Legume Source Recommended Approach

- Expression level of 2S albumin?? – soy vs. peanut
- Amino acid sequence homology with Ara h 2 from peanut??
- Ability to provoke mediator release from basophils armed with IgE antibodies of peanut-allergic individuals??

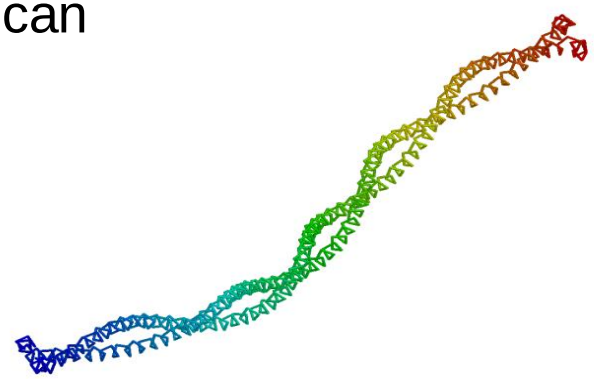
Tropomyosin Superfamily

- Tropomyosins are a family of closely related proteins present in all eukaryotic cells
  - Play a key role in regulation of muscle contraction
- Major allergens in crustacean and molluscan shellfish
  - Pen a 1 (shrimp), Cha f 1 (crab), Tod p 1 (squid), Hal d 1 (abalone), Cra g 1 (oyster), Hel as 1 (snail)
- Inhalant allergen from mites and cockroaches
  - Minor inhalant allergen in most societies



Tropomyosin Superfamily

- Only non-vertebrate tropomyosins have been identified with allergenic activity
  - Differences in the IgE binding epitopes in the C-terminal portion of tropomyosin results in no cross-reactivity to vertebrate tropomyosin
  - Of those that are allergenic, they have been shown to be highly cross-reactive
- Resistant to heat and digestion
  - Tropomyosins are water soluble and can withstand boiling



Novel Insect Source Recommended Approach

- Likely to express invertebrate tropomyosin
- Amino acid sequence homology with shrimp tropomyosin??
- Ability to provoke mediator release from basophils armed with IgE antibodies of shrimp-allergic individuals??

Conclusions & Recommendations

- Novel food sources of protein will inevitably be allergenic
- Thus, be careful about marketing these foods as non-allergenic
- It is more accurate to state that these food sources contain no commonly allergenic food
- But be careful of potential for cross-reactivity with commonly allergenic foods

Conclusions & Recommendations

- Novel food regulations in Canada and EU will require assessment of allergenic potential
- Be cautious in U.S. and other countries where the regulatory path forward on novel foods is unclear; you might simply get blocked
- Example: lupine in the U.S.

Conclusions & Recommendations

- Allergenic potential should not be a deterrent to marketing of novel food protein sources
- Clear labeling is the key
- With clear labeling, those consumers who develop allergic reactions will be able to avoid the food
- Preventive allergen controls will allow management of allergen risks within manufacturing facilities

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