3 Allergen Nomenclature

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3.1 HISTORICAL INTRODUCTION

The history of allergen nomenclature dates to the time when extracts of allergen sources were fractionated using a variety of classical biochemical separation techniques and the active (most allergenic) fraction was usually named according to the whim of the investigator. In the 1940s and 1950s, attempts were made to purify pollen and house dust allergens, using phenol extraction, salt precipitation, and electrophoretic techniques. In the 1960s, ion exchange and gel filtration media were introduced and ragweed "antigen E" was the first allergen to be purified [1]. This allergen was named so by King and Norman because it was one of the five precipitation lines (labeled A–E) that reacted with rabbit polyclonal antibodies to ragweed in Ouchterlony immunodiffusion tests. Following purification, precipitation line E, or "antigen E" was shown to be a potent allergen. Later, Marsh, working in Cambridge, England, isolated an important allergen from rye grass pollen (*Lolium perenne*) and used the name "Rye 1" to indicate that this was the first allergen purified from this species [2,3]. In the 1970s, many allergens were purified from ragweed, rye grass, insect venoms, and other sources. The field was led by the laboratory of the late Dr. David Marsh, who had moved to the Johns Hopkins University, Baltimore, Maryland. At Johns Hopkins, the ragweed allergens Ra3, Ra4, Ra5, and Ra6, and the rye grass allergens Rye 2 and Rye 3 were isolated and used for immunological and genetic studies of hay fever [4–6]. At the same time, Ohman et al. identified Cat-I, the major cat allergen [7]. Elsayed purified allergen M from codfish [8,9].

The state of the art in the early 1970s was reviewed in a chapter by Marsh in a seminal book, *The Antigens*, which described the molecular properties of allergens, the factors that influenced allergenicity, the immune response to allergens, and immunogenetic studies of immunoglobulin E (IgE) responses to purified pollen allergens [10]. This chapter provided the first clear definition of a "major" allergen, which Marsh defined as a highly purified allergen that induced immediate skin test responses in >90% of allergic individuals, in contrast to a "minor" allergen, to which <20% of patients reacted with skin test responses. Today, a major allergen is generally regarded as one to which >50% of patients with an allergy to its source react [11].

With the introduction of crossed immunoelectrophoresis (CIE) and crossed radioimmunoelectrophoresis (CRIE) for allergen identification by Løwenstein and colleagues in Scandinavia, there was a tremendous proliferation of the

number of antigenic proteins and CIE/CRIE peaks identified as allergens. Typically, 10–50 peaks could be detected in a given allergen source based on reactivity with rabbit polyclonal antibodies or IgE antibodies [6,11–13]. These peaks were given a multitude of names such as Dp5, Dp42, Ag12, and so on. Inevitably, the same allergens were referred to by different names in different laboratories; for example, mite antigen P1 was also known as Dp42 or Ag12. It was clear that a unified nomenclature was urgently needed.

3.1.1 Three Men in a Boat

The origins of the systematic allergen nomenclature can be traced to a meeting among Drs. David Marsh (at that time, Johns Hopkins University, Baltimore, MD, USA), Henning Løwenstein (at that time, University of Copenhagen, Denmark), and Thomas Platts-Mills (at that time, Clinical Research Centre, Harrow, UK) on a boat ride on Lake Constance (Bodensee), Konstanz, Germany, during the 13th Symposium of the Collegium Internationale Allergologicum in July 1980 [14]. The idea was to develop a systematic allergen nomenclature based on the Linnaean binomial nomenclature for naming all living things, with added numerals to indicate different allergens from the same source. It was decided to adopt a system whereby the allergen was named based on the first three letters of the genus and the first letter of the species (both in italics) followed by a Roman numeral to indicate the allergen in the chronological order of purification. Thus ragweed antigen E became *Ambrosia artemisiifolia* allergen I or *Amb a* I and Rye 1 became *Lolium perenne* allergen I or *Lol p* I.

An allergen nomenclature subcommittee was formed under the auspices of the World Health Organization (WHO) and International Union of Immunological Societies (IUIS) and the criteria for including allergens in the systematic nomenclature were established. These included strict criteria for biochemical purity, as well as criteria for determining the allergenic activity of the purified protein. A committee chaired by Marsh, and including Henning Løwenstein, Thomas Platts-Mills, Te Piao King (Rockefeller University, New York), and Larry Goodfriend (McGill University, Montreal, Canada) prepared a list of allergens that fulfilled the inclusion criteria and established a process for investigators to submit names of newly identified allergens. The original list, published in the Bulletin of the WHO in 1986, included 27 highly purified allergens from grass, weed, tree pollens, and house dust mites [15].

The systematic allergen nomenclature was quickly adopted by allergy researchers and proved to be a great success. It was logical, easily understood, and readily assimilated by allergologists and other clinicians who were not directly involved with the details of allergen immunochemistry. The nomenclature and allergen designations, such as *Der p* I, *Fel d* I, *Lol p* I, and *Amb a* I, were used at scientific meetings and in the literature, and expanded rapidly to include newly isolated allergens.

3.2 THE REVISED ALLERGEN NOMENCLATURE

3.2.1 Allergens

The widespread use of molecular cloning techniques to identify allergens in the late 1980s and 1990s led to an exponential increase in the number of allergens described. Many allergen nucleotide sequences were obtained by cDNA cloning or PCR amplifications and it soon became apparent that the use of Roman numerals was unwieldy, for example, *Lol p* I through *Lol p* XI [16,17]. The use of italics to denote a purified protein was inconsistent with the nomenclature used in bacterial genetics and the human leukocyte antigen (HLA) system, where italicized names denote a gene product and a regular typeface indicates expressed proteins. In 1994, the allergen nomenclature was revised so that the allergen designation was shown in regular type. Arabic numerals replaced the Roman ones. Thus *Amb a* I, *Lol p* I, and *Der p* I of the original 1986 nomenclature are now referred to as Amb a 1, Lol p 1, and Der p 1 in the current nomenclature, which has been published in several scientific journals [18–20].

3.2.1.1 Inclusion Criteria

A key part of the systematic WHO/IUIS nomenclature is that the allergen should satisfy biochemical criteria, which define the molecular structure of the protein, and immunologic criteria, which define its importance as an allergen. Originally, the biochemical criteria were based on protein purity, established for example by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, isoelectric focusing, or high-pressure liquid chromatography, and physicochemical properties including molecular weight, isoelectric point, and N-terminal amino acid sequence [20]. Today, the full nucleotide or amino acid sequence is generally required. An outline of the inclusion criteria is shown in Table 3.1. A more detailed list of requirements for the inclusion of an allergen in the WHO/IUIS nomenclature can be found in the allergen submission form (http://www.allergen.org/submission.php). An important aspect of these criteria is that the submission information should provide an unambiguous description whereby other investigators can identify the very same allergen and make comparative studies. Originally, this was achieved by purifying the protein, developing monospecific or monoclonal antibodies to it, and providing either the allergen or antibodies to other researchers for verification. Nucleotide and amino acid sequencing unambiguously identifies the allergen and enables sequence variation between cDNA clones of the same allergen definition [21–24]. Allergen preparations, sequences, and antibodies submitted for inclusion in the systematic nomenclature are expected to be made available to other investigators for research studies.

A second set of inclusion criteria involves demonstrating that the purified allergen has allergenic activity, both *in vitro* and *in vivo*. Researchers use a variety of techniques for measuring IgE antibodies *in vitro*, including radioallergosorbent-based techniques, immunoblotting, radioimmunoassays

TABLE 3.1
Allergens: Criteria for Inclusion in the WHO/IUIS Nomenclature

1. The molecular and structural properties should be clearly and unambiguously defined, including:
   - Purification of the allergenic protein to (near) homogeneity.
   - Determination of molecular weight, isoelectric point, and glycosylation pattern.
   - Determination of nucleotide and/or amino acid sequence.
   - Production of monospecific or monoclonal antibodies to the allergen.
2. The importance of the allergen in causing IgE responses should be defined by:
   - Comparing the prevalence of serum IgE antibodies in large population(s) of patients allergic to the same allergen source. Ideally, at least 50 or more patients should be tested.
   - Demonstrating allergenic activity, for example by skin testing or histamine release assay.
   - Investigating whether depletion of the allergen from an allergenic extract (e.g., by immunoabsorption) reduces its IgE binding activity.
   - Demonstrating, where possible, that recombinant allergens have IgE antibody-binding activity comparable to the natural allergen.

using labeled allergens, enzyme immunoassay (ELISA), and fluorescent enzyme immunoassay (FEIA). It is important to screen a large number of sera from an unselected allergic population to establish the prevalence of IgE reactivity. Ideally, 50 or more sera should be screened, although an allergen can be included in the nomenclature if the prevalence of IgE reactivity is >5% or if the allergen elicits IgE responses in as few as five patients.

Several methods for measuring IgE to specific allergens are available. ELISA systems allow large numbers of sera to be screened for allergen-specific IgE by using a capture monoclonal antibody to bind the allergen. Serum IgE antibodies are then allowed to bind to the immobilized allergen and are detected with biotinylated anti-IgE. A schematic for the quantification of Der p 2-specific IgE is shown in Figure 3.1A. The correlation between the results of the allergen-capture ELISA for IgE to Der p 1 and Der p 2 and the ImmunoCAP® (Thermo Fisher Scientific Inc.), the current standard for quantitative IgE measurement, is shown in Figure 3.1B. The assay was quantified using a chimeric mouse anti-Der p 2 and human IgE epsilon antibody and provided results in nanogram per milliliter of allergen-specific IgE. The allergen-capture ELISA results for IgE to Der p 1, Der p 2, and Fel d 1 correlated with the IgE measurements obtained by FEIA [25]. A streptavidin-CAP assay, a variation of the ImmunoCAP®, uses biotinylated allergens and allows IgE antibodies to specific allergens to be routinely measured by FEIA [26].

Static or suspension microarray systems also have been developed that enable simultaneous measurement of IgE antibodies to multiple allergens. Microarrays provide a profile of IgE responses to specific allergens. One commercial test uses a static allergen array on allergen-coated glass slides to measure IgE antibodies in sera to over a 100 purified natural or recombinant allergens at the same time. Results of microarray assays are as sensitive as those from FEIA with allergen extracts but the microarray requires only 30 µl of serum [27–29]. Similarly, a fluorescent multiplex suspension array technology has been developed in which allergens are covalently coupled to polystyrene microspheres containing different ratios of fluorescent dyes. Each microsphere bead can be distinguished by laser flow cytometry and forms a solid phase to which IgE antibodies bind and can be detected using biotinylated anti-IgE and streptavidin phycoerythrin. This fluorescent microarray [30] currently measures total IgE and specific IgE to 11 purified allergens simultaneously, using only 20 µl of serum. Array technologies are suited, especially to large population surveys or birth cohorts for monitoring IgE responses to multiple allergens and for pediatric studies where sera are often in short supply.

Demonstrating that a protein has allergenic activity *in vivo* is important, especially because many allergens are now produced as recombinant molecules before the natural allergen is purified (if ever). Several mite, cockroach, and fungal allergens (e.g., *Aspergillus*, *Alternaria*, and *Cladosporium*) have been defined solely using recombinant proteins and it is unlikely that much effort will be directed to isolating their natural counterparts. Ideally, the allergenic activity of recombinant proteins should be confirmed *in vivo* by quantitative skin testing or *in vitro* by histamine release assays. Skin testing studies were carried out using a number of recombinant allergens, including Bet v 1, Asp f 1, Bla g 4, Bla g 5, Der p 2, Der p 5, and Blo t 5 [22,31,32]. The recombinant proteins showed potent allergenic activity and gave positive skin tests at the picogram level.

3.2.1.2 Resolving Ambiguities in the Nomenclature

Early on it was recognized that because the allergen nomenclature system was based on the Linnaean genus and species names and some shared the same first three letters of the genus and/or the first letter of the species name, some unrelated allergens would receive the same allergen designation: *Candida* allergens could be confused with dog allergen (*Canis domesticus*); there were multiple related species of *Vespula* (Vespid) allergens; and the *Periplaneta americana* (American cockroach) allergen had to be distinguished from that of *Persea americana* (avocado). These ambiguities were overcome by adding a further letter to either the genus or species name. Examples are Cand a 1 for *Candida albicans* allergen 1 and Can f 1 for dog (*Canis familiaris*) allergen 1; Ves v 1 or Ves vi 1, to indicate *Vespula vulgaris* or *V. vidua* allergens, respectively; Per a 1 or Pers a 1 for the respective cockroach or avocado allergen. Many allergens had biochemical names that described their biological function and which were assigned before the allergen nomenclature was conceived. Examples include egg allergens (ovomucoid and ovalbumin); insect allergens (phospholipases and hyaluronidases); and tropomyosins

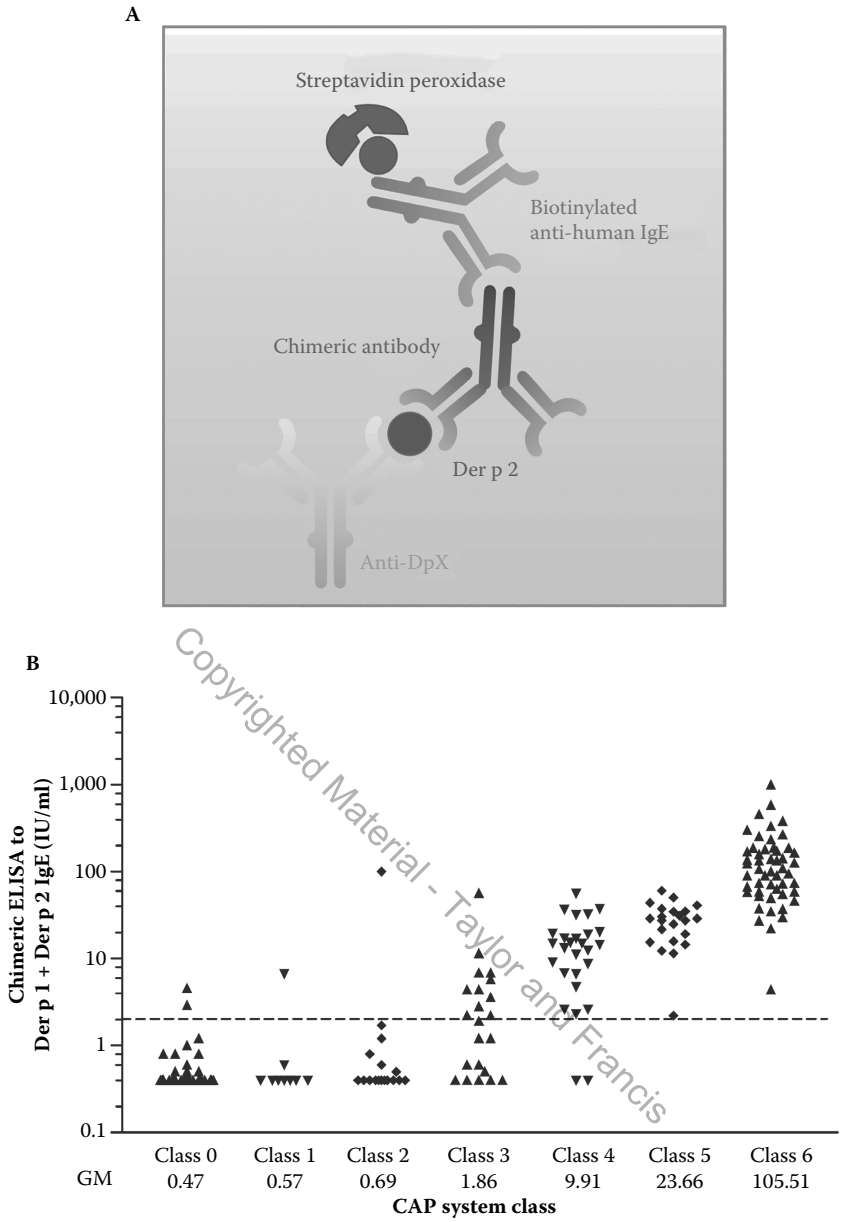

FIGURE 3.1 **(See color insert.)** Chimeric ELISA for measuring allergen-specific IgE. (**A**) Schematic graphic of the ELISA. Microtiter plates are coated with a monoclonal anti-Der p 2 antibody followed by the allergen and incubated with a chimeric mouse Fab/human Fcε anti-Der p 2 antibody or patient's serum. The chimeric and the IgE antibodies that bind to the immobilized allergen are detected using biotinylated anti-IgE and streptavidin peroxidase. The chimeric antibody is used to generate a control curve and IgE values for patients' sera are interpolated from this curve. (**B**) Correlation between the chimeric ELISA for IgE to Der p 1 and Der p 2 and FEIA (ImmunoCAP®) for measuring IgE to house dust mite. There was an excellent quantitative correlation between the ELISA and FEIA results for 212 sera from patients with asthma, wheezing, and/or rhinitis ($r = .86$, $p < .001$). (Reproduced from Trombone, AP et al. *Clin Exp Allergy* 2002; 32: 1323–1328. With permission.) *Abbreviations:* CAP, ImmunoCAP®; GM, Geometric mean.

from shrimps, mites, and cockroaches. Sequence homology searches have assigned allergens to particular protein families and have provided important clues to their biological function. To some extent, allergens segregate among protein families according to whether they are indoor allergens, outdoor allergens, plant or animal food allergens, or injected allergens:

- Indoor allergens (e.g., animal dander; fecal particles from mites and cockroaches; mold spores):
  - Proteolytic enzymes (serine and cysteine proteases), lipocalins (ligand-binding proteins), tropomyosins, albumins, calcium-binding proteins, and protease inhibitors [22,33].
- Outdoor allergens (e.g., pollens from grasses, trees, and weeds; mold spores):
  - Plant pathogenesis-related (PR-10) proteins, pectate lyases, β-expansins, calcium-binding proteins (polcalcins), defensin-like proteins, and trypsin inhibitors [21,23,34,35].

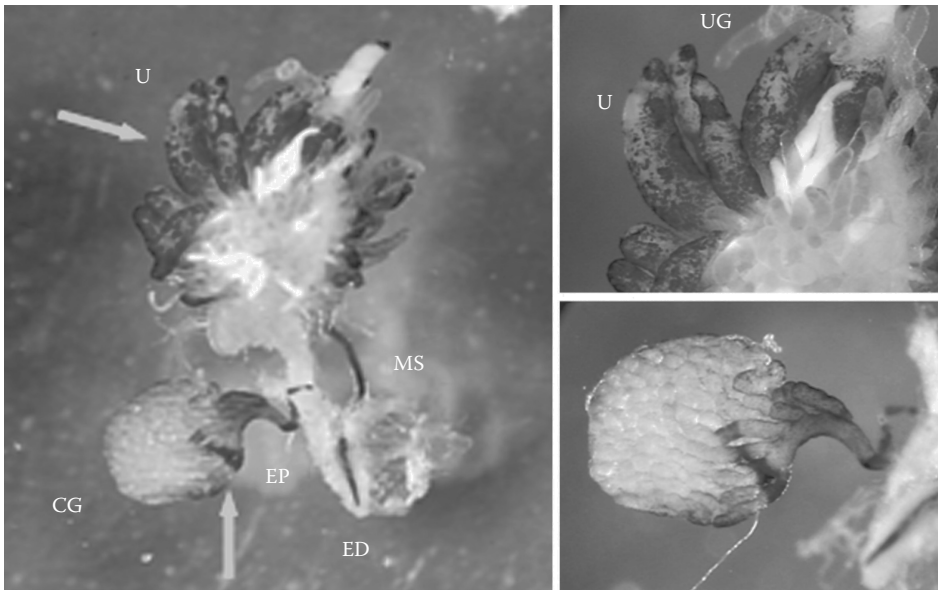

FIGURE 3.2 (See color insert.) Tissue localization of German cockroach allergen Bla g 4 mRNA in the large apical utricles (U) and the base of the conglobate gland (CG) of the male reproductive system by *in situ* hybridization (left panel). Right panel shows higher magnification. Bla g 4 mRNA is found only in the male accessory reproductive glands and is transferred to the female during copulation. (Reproduced from Fan, Y et al. *Insect Mol Biol* 2005; 14: 45–53. With permission.)

- Plant or animal food allergens (e.g., fruits, vegetables, nuts, milk, eggs, shellfish, and fish):
  - Lipid transfer proteins, profilins, seed storage proteins, lactoglobulins, caseins, tropomyosins, and parvalbumins [36–38].
- Injected allergens (e.g., insect venoms and some therapeutic proteins):
  - Phospholipases, hyaluronidases, pathogenesis-related proteins, and asparaginase [39,40].

Allergens belonging to these protein families have biologic functions that are important to the organism that is the allergen source. Proteolytic enzymes are involved in digestion, tropomyosins and parvalbumins in muscle contraction, and profilins in actin polymerization in plants. The mouse lipocalin allergen, Mus m 1, is produced in the liver of male mice, secreted in large amounts in the urine, and serves to mark the territories of male mice [41]. The cockroach allergen Bla g 4, a lipocalin homolog, is produced in the accessory glands of the male reproductive system and is speculated to bind pheromones (Figure 3.2) [42,43]. Crystallographic studies show that Bet v 1, a plant pathogenesis-related (PR-10) protein, contains a hydrophobic pocket that could bind brassinosteroids and hence may function as a plant steroid carrier [44].

In the allergy literature, it is preferable to use the systematic allergen nomenclature. However, in other contexts, such as comparisons of biochemical activities or protein structure, it may be appropriate or more useful to use the biochemical names. A selected list of the allergen nomenclature and biochemical names of inhalant, food, and venom allergens is shown in Table 3.2. There are now over 90 three-dimensional allergen structures in the Protein Database (http://fermi.utmb.edu/SDAP) and allergens are found in approximately 250 domains of the currently defined 14,831 protein families in the Pfam protein family database (http://pfam.sanger.ac.uk). Thus allergens are represented by a mere 1.7% of the Pfam domains, which are equivalent to approximately 190 protein families representing a fair degree of diversity at both structural and biological levels. Such diversity is likely to preclude the existence of a few common structural features, for example, amino acid sequence motifs or protein structures, which predispose proteins to act as allergens [21,24].

3.2.2 Isoallergens, Isoforms, and Variants

Originally, isoallergens were broadly defined as multiple molecular forms of the same allergen, sharing extensive antigenic (IgE) cross-reactivity. The revised nomenclature defines an isoallergen as an allergen from a single species, sharing a similar molecular size, identical biological function, and ≥67% amino acid sequence identity [8]. A two-digit number, following the dot after the number given to the allergen, designates the isoallergen. Some allergens, which were previously included into the nomenclature as separate entities, share extensive sequence identities and some antigenic cross-reactivity, but were named independently as they do not meet all the criteria to be classified as isoallergens. Examples include Lol p 2 and Lol p 3 (65% identity) and Amb a 1 and Amb a 2 (65% identity).

The designation "Group" is still used to describe structurally related allergens from different species within the same genus, or from closely related genera. In these cases, the levels of amino acid sequence identity can range from as little

TABLE 3.2
Molecular Properties of Common Allergens

Source	Allergen	MW (kDa)	Homology/Function
Inhalants			
Indoor			
House dust mite (*Dermatophagoides pteronyssinus*)	Der p 1	25	Cysteine protease[a]
	Der p 2	14	MD-2-related lipid-recognition domain family member[a]
	Der p 3	30	Serine protease
Cat (*Felis domesticus*)	Fel d 1	36	Secretoglobin[a]
Dog (*Canis familiaris*)	Can f 1	25	Lipocalin
Mouse (*Mus musculus*)	Mus m 1	21	Lipocalin (territory marking protein)[a]
Rat (*Ratus norvegicus*)	Rat n 1	21	Pheromone-binding lipocalina
Cockroach (*Blatella germanica*)	Bla g 2	36	Inactive aspartic protease[a]
Outdoor			
Pollen—grasses			
Rye (*Lolium perenne*)	Lol p 1	28	β-expansin
Timothy (*Phleum pratense*)	Phl p 5	32	Putative ribonuclease[a]
Bermuda (*Cynodon dactylon*)	Cyn d 1	32	β-expansin
Pollen—weeds			
Mugwort (*Artemisia vulgaris*)	Art v 1	28	Defensin-like protein[a]
Ragweed (*Ambrosia artemisiifolia*)	Amb a 1	38	Pectate lyase
Pollen—trees			
Birch (*Betula verrucosa*)	Bet v 1	17	Pathogenesis-related protein[a]
Foods			
Cow's milk (*Bos domesticus*)	Bos d 5 (β-lactoglobulin)	18	Lipocalin[a]
Hen's egg (*Gallus domesticus*)	Gal d 1 (ovomucoid)	28	Trypsin inhibitor
Codfish (*Gadus callarias*)	Gad c 1	12	Calcium-binding muscle protein (parvalbumin)
Peanut (*Arachis hypogaea*)	Ara h 1	63	Vicilin seed storage protein[a]
Venoms			
Bee (*Apis mellifera*)	Api m 1	19.5	Phospholipase A_2[a]
Wasp (*Polystes annularis*)	Pol a 2	38	Hyaluronidase
European hornet (*Vespa crabro*)	Vesp c 1	34	Phospholipase A_1B
Fire ant (*Solenopsis invicta*)	Sol i 1	18	Phospholipase A_1B
Fungi			
Aspergillus fumigatus	Asp f 1	18	Cytotoxin (mitogillin)[a]
Alternaria alternata	Alt a 10	53	Aldehyde dehydrogenase
Latex			
Rubber tree (*Hevea brasiliensis*)	Hev b 1	14	Rubber elongation factor
	Hev b 2	34	β-1,3-glucanase

[a] Allergens of known three-dimensional structure.

as 40% to approximately 90%. Similarities in tertiary structure and biologic function are also taken into account when describing allergen groups. Examples include the Group 2 mite allergens (Der p 2, Der f 2 and Lep d 2, Gly d 2, and Tyr p 2) showing 40% to 88% identity, and the Group 5 ragweed allergens (Amb a 5, Amb t 5, and Amb p 5) showing approximately 45% identity. The *Dermatophagoides* Group 2 allergen structures have been determined by x-ray crystallography and nuclear magnetic resonance spectroscopy. The structures of Group 2 allergens from other mite species were modeled on the *Dermatophagoides* Der p 2 structure (Figure 3.3). This enabled the structural basis for antigenic relationships between members of the group to be defined [20–22].

The term "variant" or "isoform" is used to indicate allergen sequences that differ from each other by only a limited number of amino acid substitutions (i.e., polymorphic variants of the same allergen). Typically, variants may be identified by sequencing several cDNA clones of a given allergen. Variants have been reported for Der p 1, Der p 2, Amb a 1,

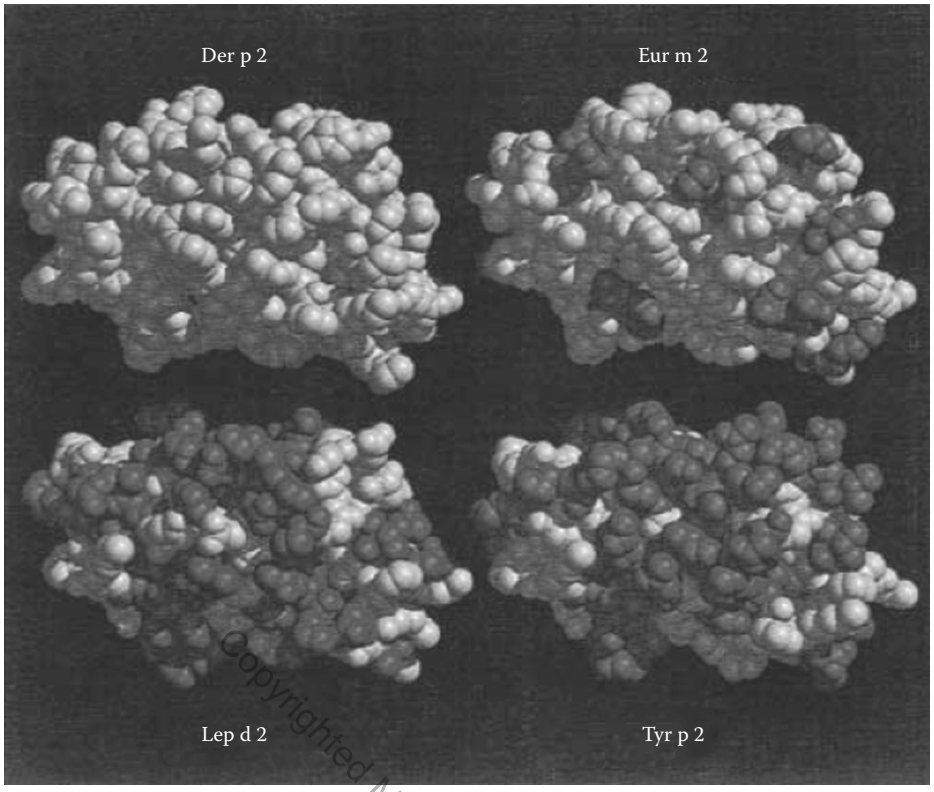

FIGURE 3.3 (**See color insert.**) Space-filling models of Group 2 allergens from house dust mite. Amino acid substitutions are shown in gray scale. The space-filling model of Der p 2 was generated from nuclear magnetic resonance spectroscopy studies and has subsequently been confirmed by x-ray crystallography [22]. Eur m 2 shows 85% sequence identity with Der p 2 and seven of the substituted amino acids are shown in gray on the surface structure. There is extensive cross-reactivity between Der p 2 and Eur m 2 [61]. In contrast, Lep d 2 and Tyr p 2 show only 40% amino acid identity with the other Group 2 allergens [62]. They show many substitutions on the antigenic surface of the molecules and have limited antigenic cross-reactivity for monoclonal antibody and human IgE. (Reproduced from Smith, AM et al. *J Allergy Clin Immunol* 2001; 107: 977–984; Gafvelin, G et al. *J Allergy Clin Immunol* 2001; 107: 511–518. With permission.)

Cry j 1, and, for the most prolific Bet v 1, 42 sequences have been deposited in the GenBank database. Isoallergens and variants are denoted by the addition of four numeral suffixes to the allergen name. The last two numerals, positions 3 and 4 after the dot, distinguish between allergen variants and polymorphic isoforms.

One of the tasks of the IUIS allergen nomenclature subcommittee is a regular reevaluation process of the data deposited in the nomenclature database. Many allergens recorded in the IUIS allergen database were originally submitted with partial or missing sequence data. Later on, full sequences became available, which in some cases led to inconsistent allergen numbers. Therefore, the database has to be screened periodically and allergen designations need to be corrected from time to time. The ragweed (*Ambrosia artemisiifolia*) pollen allergens Amb a 1 and Amb a 2 both belong to the pectate lyase family. Amb a 2 isoallergens showed 61% to 70% sequence identity to Amb a 1 isoallergens and were hence renamed as Amb a 1.05. Entries for the larvae of the nonbiting midge *Chironomus thummi thummi* contained nine allergens (Chi t 1–Chi t 9) with 16 variants, all of which are hemoglobin subunits with sequence identities between 28% and 99%. As Chi t 5 through Chi t 8 showed sequence identities >50% to Chi t 3 they received new designations as Chi t 3 isoallergens. This renaming was based on more detailed phylogenetic analyses of the proteins that showed a clear grouping of Chi t 5 through Chi t 8 with Chi t 3. The recommended 67% sequence identity for two allergens to be designated as isoallergens is only a guide.

The Group 1 allergens from tree pollen have an unusually high number of isoallergens and variants. The Bet v 1 sequences that show 73% to 98% sequence homology are named Bet v 1.01 through Bet v 1.30. At the time of this writing (11/2012), the Bet v 1 allergen nomenclature was under intense discussion that will likely result in a substantial reduction of the number of isoforms. The Group 1 allergen from hornbeam (*Carpinus betulus*), Car b 1, has three isoallergens, which show 74% to 88% identity (Car b 1.01, 1.02, and 1.03) and the nomenclature committee's most recent records show 16 sequences of Car b 1. Four isoallergens of the hazel pollen allergen, Cor a 1, with 10 sequences have also been recorded. The reasons for the Group 1 tree pollen allergens having so many variants are unclear. Latex provides another example of distinctions in nomenclature.

The 20 kDa hevein precursor or prohevein, Hev b 6.01, gives rise to the most important 5 kDa latex allergen hevein, designated as Hev b 6.02. It is one of the two fragments derived from Hev b 6.01, the other being Hev b 6.03, the 14 kDa C-terminal fragment. This is one of the few examples where the two digits behind the dot were used to identify fragments derived from one protein rather than isoforms. Studies have also uncovered a prodigious number of isoforms among mite Group 1 and Group 2 allergens. High-fidelity PCR sequencing of environmental isolates of dust mites revealed 23 isoforms of Der p 1 and 13 isoforms of Der p 2 [24]. Because isoforms differ by only a few amino acid residues, analysis of immunoreactivity to isoforms can be useful in defining antibody-binding sites and T-cell epitopes on allergens [45].

3.3 NOMENCLATURE FOR ALLERGEN GENES AND RECOMBINANT OR SYNTHETIC PEPTIDES

In the revised nomenclature, italicized letters are reserved to designate gene coding for allergens. Two genomic allergen sequences have been determined from animal dander allergens: cat allergen, Fel d 1 and mouse urinary allergen, Mus m 1. Fel d 1 has two separate genes encoding chain 1 and chain 2 of the molecule, which are designated as *Fel d 1A* and *Fel d 1B,* respectively [24]. Genomic sequences of Bet v 1, Cor a 1, and an apple PR-10 family member ("Mal d 1") have also been determined [46,47]. Mal d 1 is an example of an incomplete or nonsensitizing allergen, that is, an allergen that can interact with preformed Bet v 1-specific IgE antibodies, but is unable to induce the production of IgE. Thus, symptoms of the pollen food syndrome in birch pollen allergic patients who eat apples are due to IgE cross-reactivity between Bet v 1 (the primary sensitizer) and Mal d 1 (with which the IgE anti-Bet v 1 interacts).

When recombinant allergens were first introduced, researchers often used the term "native allergen" to distinguish the natural protein from the recombinant allergen. However, because "native" has implications for protein structure (i.e., native conformation), it was decided that the term "natural allergen" should be used to indicate any allergen purified from a natural source material. Natural allergens may be denoted by the prefix (n) to distinguish them from recombinant allergens, which are indicated by the prefix (r) before the allergen name (e.g., nBet v 1 and rBet v 1). No distinction is made between recombinant allergens produced in bacterial, yeast, or mammalian expression systems. Synthetic peptides are indicated by the prefix (s), with the particular peptide residues indicated in parentheses after the allergen name. Thus a synthetic peptide encompassing residues 100 to 120 of Bet v 1.0101 would be indicated: sBet v 1.0101 (100–120). At such a detailed level, the nomenclature, while technically sound, begins to become cumbersome and rather long-winded for most purposes. There are also additional refinements to the nomenclature which cover substitutions of different amino acid residues within synthetic peptides. This aspect of the nomenclature (which is based on that used for synthetic peptides of immunoglobulin sequences) is detailed in the revised nomenclature document to which aficionados are referred for full details [20].

3.4 FUTURE PERSPECTIVES OF ALLERGEN NOMENCLATURE

3.4.1 Protein Family Membership

When the cDNA of the major birch pollen allergen Bet v 1 was published in 1989 as the first nucleotide sequence of a plant allergen [48], it became clear that this birch pollen protein belonged to a family of proteins. As more and more members of this family were discovered, the emerging superfamily of proteins was eventually named after Bet v 1 [49]. Today, the Bet v 1-like superfamily of proteins contains 14 families and 23,609 member proteins from 4,418 species distributed across all superkingdoms of life (http://pfam.sanger.ac.uk/clan/CL0209). In general, the steadily increasing number of allergen sequences allows the study of the evolutionary biology of allergens [50] and the determination of their distribution into protein families [23,51]. The AllFam database (http://www.meduniwien.ac.at/allergens/allfam) provides information about the protein family membership of allergens. Interestingly, all known allergens belong to only 1.7% of the 14,831 protein families described in version 27.0 (3/2013) of the Pfam database (http://pfam.sanger.ac.uk) [52]. Although the current official allergen nomenclature is directly linked to the source organism, it provides no information on the protein family membership of allergens. This is not surprising as the classification of allergens by protein family membership has only become available in more recent years and its implications on allergen nomenclature were first discussed in 2008 [53]. Initially, the same allergen numbers were given to the same type of proteins, a procedure that could not be completely adhered to as the number of allergens rapidly increased. Hence, the Bet v 1 homologs or profilins could not be assigned identical numbers in all sources (Table 3.3). However, the grouping of allergens by protein families provides valuable information on possible cross-reactions for researchers as well as clinicians. Der p 1 from *Dermatophagoides pteronyssinus* and Der f 1 from *D. farinae* belong to the protein family of cysteine proteases. Both proteins are potent cross-reactive mite allergens. Chruszcz and colleagues were able to determine a common epitope on Der p 1 and Der f 1 by resolving the crystal structures of both natural allergens in complex with a monoclonal antibody [54]. While indicating possible cross-reactivities, it seems advisable to link the allergen nomenclature database to a more specialized database, such as the AllFam, that can provide the information on protein family memberships of allergens. Very recently, the high-resolution structure of the *Alternaria alternata* allergen, Alt a 1, became available [55]. Alt a 1 is a unique β-barrel composed of 11 β-strands and forms a butterfly-like dimer linked by a disulfide bond. This structure has no equivalent in the Protein Data Bank and, hence, Alt a 1 is the founding member of a new protein family.

TABLE 3.3
Protein Family Membership and Allergen Designations

Allergen Source	PR-10 Family	Profilin Family
Birch (*Betula verrucosa*)	Bet v 1	Bet v 2
Apple (*Malus domestica*)	Mal d 1	Mal d 4
Celery (*Apium graveolens*)	Api g 1	Api g 4
Kiwi (*Actinidia deliciosa*)	Act d 8	Act d 9
Peanut (*Arachis hypogaea*)	Ara h 8	Ara h 5
Soybean (*Glycine max*)	Gly m 4	Gly m 3

3.5 THE IUIS SUBCOMMITTEE ON ALLERGEN NOMENCLATURE

Allergens to be considered for inclusion in the nomenclature are reviewed by the IUIS allergen nomenclature subcommittee, which is currently chaired by Dr. Heimo Breiteneder (Medical University of Vienna, Austria) and has 20 members from all over the world (Table 3.4). The committee meets annually at an international allergy/immunology meeting and discusses new proposals it has received during the year, together with any proposed changes or additions to the nomenclature and the database. There is also a committee-at-large, which is open to any scientist with an interest in allergens, to whom decisions made by the subcommittee are circulated. The procedure for submitting candidate names for allergens to the subcommittee is straightforward. Having purified the allergen, determined its sequence, and shown that it reacts with IgE from individuals who are allergic to its source investigators should download the allergen submission form from the nomenclature committee website (http://www.allergen.org/submission.php) and send the completed form to the subcommittee prior to publishing articles describing the allergen. The subcommittee will provisionally accept the author's suggested allergen name, or assign an allergen a name, provided that the inclusion criteria are satisfied. The name will later be confirmed at a full meeting of the subcommittee. Occasionally, the subcommittee has to resolve differences between investigators who may be using different names for the same allergen, or disputes concerning the chronological order of allergen identification. These issues can normally be resolved by objective evaluation of each case.

3.5.1 Allergen Databases

The official website for the WHO/IUIS Subcommittee on Allergen Nomenclature is http//:www.allergen.org. This site lists all allergens and isoforms that are recognized by the subcommittee and is updated on a regular basis. Over the past several years, a number of other allergen databases have been generated by academic institutions, research organizations, and industry-sponsored groups (Table 3.5). These sites differ in their focus and emphasis but are useful sources of information about allergens. The Structural Database of Allergenic Proteins (SDAP) was developed at the Sealy Center for Structural Biology, University of Texas Medical Branch, and provides detailed structural data on allergens in the WHO/IUIS nomenclature, including sequence information and Protein Data Bank (http://www.rcsb.org) files and programs to analyze IgE epitopes. Amino acid and nucleotide sequence information are also compiled in the SWISS-PROT (http://www.ebi.ac.uk/uniprot) and NCBI (http://www.ncbi.nlm.nih.gov/guide/all) databases.

The Food Allergy Research and Resource Program (FARRP) and InformAll databases focus on food allergens and provide sequence similarity searches (FARRP) and clinical data (skin tests and provocation tests) on food allergens (InformAll). The InformAll food allergen database is in the process of being moved (11/2012) from its current location at the Institute of Food Research, Norwich, UK, most likely to the University of Manchester. The Allergome database provides regular updates on allergens from publications in the scientific literature. The AllFam database, which was developed based on allergen information in the Allergome database and the data on protein families from the Pfam database, contains all allergens with known sequences that can be assigned to at least one Pfam family. The database is maintained by Drs Breiteneder and Radauer at the University of Vienna and can be accessed at http://www.meduniwien.ac.at/allergens/allfam.

3.6 CONCLUDING REMARKS

The three men in a boat did a remarkably good job. The use of the systematic allergen nomenclature has been extremely successful, has significantly enhanced research in the area, and continues to be revised and updated. The use of the generic terms "major" and "minor" allergen continues to evoke discussion. Relatively few allergens fulfill the criteria originally used by Marsh to define a major allergen, (i.e., an allergen that causes IgE responses in ≥90% of allergic patients, such as Bet v 1, Fel d 1, Der p 2, and Lol p 1) [10]. However, a large number of allergens cause sensitization in >50% of patients and Løwenstein used this figure of 50% to define major allergens in the early 1980s [6]. Scientists like

TABLE 3.4
The World Health Organization and International Union of Immunological Societies Subcommittee on Allergen Nomenclature, 2006–2012[a]

Name	Institution	Country
Heimo Breiteneder PhD, Chairman	Medical University of Vienna	Vienna, Austria
Richard Goodman PhD, Secretary	University of Nebraska	Lincoln, NE, USA
Wayne R. Thomas PhD, Past Chair	TVW Telethon Institute for Child Health	Perth, Australia
Naveen Arora PhD	Institute of Genomics and Integrative Biology	Delhi, India
L. Karla Arruda MD, PhD	School of Medicine of Ribeirão Preto, University of São Paulo	Ribeirão Preto, Brazil
Martin D. Chapman PhD	INDOOR Biotechnologies, Inc.	Charlottesville, VA, USA
Fatima Ferreira PhD	University of Salzburg	Salzburg, Austria
Viswanath P. Kurup PhD	Medical College of Wisconsin	Milwaukee, WI, USA
Jørgen N. Larsen PhD	ALK-Abelló	Hørsholm, Denmark
Jonas Lidholm PhD	Phadia, AB	Uppsala, Sweden
Kåre Meno PhD	ALK-Abelló	Hørsholm, Denmark
Andreas Nandy PhD	Allergopharma Joachim Ganzer KG	Reinbek, Germany
Anna Pomés PhD	INDOOR Biotechnologies, Inc.	Charlottesville, VA, USA
Thomas A.E. Platts-Mills MD, PhD	University of Virginia	Charlottesville, VA, USA
Christian Radauer PhD	Medical University of Vienna	Vienna, Austria
Monika Raulf-Heimsoth	Ruhr University Bochum	Bochum, Germany
Marianne van Hage MD, PhD	Karolinska Institute	Stockholm, Sweden
Ronald van Ree PhD	Academic Medical Centre	Amsterdam, The Netherlands
Stefan Vieths PhD	Paul Ehrlich Institute	Langen, Germany

[a] Past Chairs of the Committee: David G. Marsh PhD (1980–1989); Te Piao King PhD (1990–1994); Henning Løwenstein PhD (1994–1997); Wayne R. Thomas PhD (1997–2006). Henning Løwenstein PhD, DMSc (Hørsholm, Denmark) is an Emeritus member of the committee.

TABLE 3.5
Allergen Databases

Database	Host	Curation	URL
WHO-International Union of Immunological Societies	University of Nebraska, Lincoln, NE, USA	Expert panel reviews submissions of new allergens; the only body officially be able to give allergen designations	http://www.allergen.org
Structural Database of Allergen Proteins (SDAP)	University of Texas, USA	Curated by host scientists with oversight by an expert review panel	http://fermi.utmb.edu/SDAP/sdap_ver.html
Allergen Online	University of Nebraska, Food Allergy Research and Resource Program, USA	Reviewed by an expert panel with annual database updates	http://allergenonline.com
InformAll	Institute of Food Research, UK	Curated by host scientists with oversight by an expert review panel	http://www.foodallergens.info
Allergome	Allergy Data Laboratories s.c., Italy	Not defined	http://www.allergome.org
The Allergen Database	The Food and Environment Research Agency	Not defined	http://www.csl.gov.uk/allergen
AllFam	Medical University of Vienna, Austria	Curated by host scientists	http://www.meduniwien.ac.at/allergens/allfam

to describe their allergen as "major" because it is effective in promoting their research and carries some weight in securing research funding. The question continues to be "What defines a major allergen?" Demonstrating a high prevalence of IgE-mediated sensitization and that the protein has allergenic activity is a minimal requirement, given the increasing sensitivity of assays to detect IgE antibodies. The contribution of an allergen to the total potency of an allergen extract should be considered (e.g., by absorption studies), as well as the amount of IgE antibody directed against the allergen, compared to other allergens purified or cloned from the same source. Other criteria include whether the allergen induces strong T-cell responses and, for indoor allergens, whether it is a suitable marker of exposure in house dust and air samples.

It is clear from many studies that some allergens play a pre-eminent role in causing immune responses in atopic individuals, are better marker proteins for immunologic, clinical, and epidemiologic studies, and are usually considered to be high-profile targets for allergy diagnostics and therapeutics. Table 3.6, which was developed together with Dr. Rob Aalberse (University of Amsterdam), lists the eight criteria for defining the properties of these "allergens that make a difference." Examples of allergens and their sources that we consider to fulfill most of these criteria are as follows:

Mite	Group 1 and Group 2 (*Dermatophagoides* sp) allergens
Animal	Fel d 1, Mus m 1, Rat n 1
Tree pollen	Bet v 1 (and structurally homologous allergens); Ole e 1
Grass pollen	Phl p 1, Phl p 5
Weed pollen	Amb a 1
Peanut	Ara h 1, Ara h 2
Shellfish	Pen a 1 and other tropomyosins from shellfish
Insect	Api m 1 (and homologous insect venom allergens)

Some of these recombinant allergens have already been shown to be effective as vaccines in clinical trials (Phl p 1 and Phl p 5) and most of the other allergens listed are being used as targets for vaccine development [4,22,56,57]. Recombinant Bet v 1 and recombinant Phl p 5a were produced under Good Manufacturing Practice and recently established as reference standards in the European Pharmacopeia for the determination of the respective proteins both in natural allergen extracts as well as in recombinant allergen products [58]. The standards adopted to determine the presence and concentrations of allergens are also widely used in environmental exposure assessments and for measuring the allergen content of vaccines. To this end, more well-defined purified allergen standards are being developed. In 2012, eight purified natural allergens were formulated into a single multiallergen standard containing the major allergens of mite, cockroach, cat, dog, mouse, and rat [59,60].

For most purposes, allergists need only be familiar with the nomenclature for allergens, rather than isoallergens, isoforms, peptides, etc. However, as measurements of allergens in diagnostics and vaccines and in environmental exposure assessments are becoming a routine part of the care of allergic patients, allergists will need to understand more about the structure and functions of allergens and how to distinguish them. Having a systematic nomenclature is an important part of this process. The systematic nomenclature is a proven success and is versatile enough to evolve with advances in molecular biology and proteomics that will continue to occur.

SALIENT POINTS

1. A systematic nomenclature for all allergens that cause disease in humans has been formulated by a subcommittee of the WHO and the International Union of Immunological Societies.
2. Allergens are described using the first three or four letters of the genus, followed by one or two letters for the species and an Arabic numeral to indicate the chronological order of allergen purification (e.g., *Dermatophagoides pteronyssinus* allergen 1 = Der p 1) or their protein family membership.
3. To be included in the systematic nomenclature, allergens have to satisfy the criteria of biochemical purity and criteria to establish their allergenic importance. It is important that the amino acid sequence of an allergen is defined without ambiguity and its allergenic activity is demonstrated in a large, unselected population of allergic patients who are exposed to the allergen.

TABLE 3.6
Eight Criteria for Defining Allergens That Make a Difference

1. A sensitization rate of >80% (>2 ng allergen-specific IgE/ml) in a large panel of allergic patients.
2. A significant proportion of total IgE (>10%) can be allergen specific.
3. Removal of an allergen from the source material significantly reduces the potency of the extract.
4. Absorption of a serum with a purified allergen significantly reduces the specific IgE to the allergen extract.
5. An allergen accounts for a significant proportion of the extractable protein in the source material.
6. An allergen can be used as a marker for environmental exposure assessment.
7. Both antibody and cellular responses to an allergen can be measured in a high proportion of allergic patients.
8. Allergens have been shown to be effective as part of allergy vaccines.

4. Modifications of the nomenclature are used to identify isoallergens, isoforms, allergen genes, recombinant allergens, and synthetic peptides. For example, Bet v 1.01 is an isoallergen of Bet v 1 and Bet v 1.0101 is an isoform or a variant of the Bet v 1.01 isoallergen. Allergen genes are denoted by italics; for example, *Fel d 1A* and *Fel d 1B* are the genes encoding chain 1 and chain 2 of Fel d 1, respectively.

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