

does not inhibit translation when this same EF-Tu mutant is the only EF-Tu available for protein synthesis. Thus, this study shows that Doc is toxic and inhibits translation because it phosphorylates and inactivates the cellular supply of EF-Tu (**Fig. 1a**).

But how is this 'switch' in enzymatic activity achieved? Recently, an extended motif was identified in a subset of Fic domains that carry out adenylation, HxFx(D/E)GNGRxRR, where the three backbone amide nitrogens of GNG bind the α - and β -phosphates of the bound nucleotide¹⁰. Critically, only glycines are allowed in these positions, as the side chains of all of the other amino acids would clash with the bound nucleotide. In Doc, the corresponding sequence is HIFNDANKRTAL. The presence of an alanine and lysine in the positions normally occupied by glycines in adenylation-specific Fic domains suggests that Doc binds its nucleotide substrate in a non-adenylation-competent manner. This is exactly what the authors show (**Fig. 1b**). Using NMR spectroscopy and the Rosetta ligand docking program, they show that the adenosine base of ATP binds near residue Tyr20 (Doc helix α 1) rather than Thr106 (Doc helix α 5), with the phosphate tail extending back toward the catalytic histidine. Notably, rather than clashing with the nucleotide base (as it would if ATP bound Doc in an adenylation-competent manner), here Lys73 forms electrostatic interactions with and stabilizes the phosphate tail (**Fig. 1c**).

So how does the antitoxin Phd inhibit Doc enzymatic activity? It is well known that the C-terminal helix of Phd (residues 50–73) is sufficient to inhibit Doc-mediated

toxicity. Superposition of the Doc–Phd complex with the Doc–ATP model reveals that the Phd C-terminal helix (known as α_{inh} in the Fic family; Doc belongs to Fic subclass 1, in which α_{inh} is provided by its cognate antitoxin¹⁰) docking site on Doc overlaps with that of ATP. Thus, when Doc is bound to Phd, it cannot bind the nucleotide, rendering it enzymatically inactive and resulting in robust translation. Together, this study not only provides a detailed molecular understanding of Doc-mediated toxicity but also clearly expands the variety of phosphotransferase activities that can be attributed to Fic domain-containing proteins.

Interestingly, the substrate orientation seen by Castro-Roa *et al.*⁴ is similar to that observed for CDP-choline in the Fic-containing protein AnkX⁹ (**Fig. 1b**). This observation is notable because AnkX, like Doc, catalyzes a phosphotransfer reaction that results in the release—rather than the transfer—of the 5' nucleotide. Consistent with this, the sequence of the corresponding motif in AnkX, like Doc, is also not adenylation specific: HPFRDANGRTFV. This motif may therefore point more generally to Fic proteins that display noncanonical functions; more broadly, the substrate reorientation seen in Doc provides an unexpected example by which enzymes can generate diversity in their reaction mechanisms.

Critically, there are still unanswered questions; namely, how is the phosphorylation of EF-Tu reversed, and how is Doc inactivated so the dormant cell can be 'revived'? Although the authors show that in the presence of ADP or GDP, the phosphorylation of EF-Tu can be reverted

by Doc, it is also possible that EF-Tu dephosphorylation is carried out by a specialized phosphatase whose identity is currently unknown. In addition, the role of proteolysis, conditional cooperativity or both in the inactivation of Doc is still not well understood. Answers to these questions will allow us to understand how toxin (Doc)-mediated growth arrest is reverted and will provide the necessary insights for the development of an entirely new class of antibiotics.

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IMMUNOLOGY

Allergy's Achilles' heel?

Allergic reactions to otherwise innocuous substances involve a complex interplay of molecular interactions—some strong, some weak. This study reveals a key role for low-affinity antibodies and thus a possible point of weakness that may be exploited for therapeutic intervention.

Brian J Sutton

Allergic reactions are inappropriate, sometimes fatal responses of our immune system involving antibodies of a class known as immunoglobulin E (IgE) that bind the allergen and trigger inflammation. Most allergens are proteins, and although we don't understand why the incidence of allergic diseases such as asthma and food allergies has risen so dramatically

in recent decades, we do now know about many of the proteins involved. For example, anaphylactic reaction to peanuts, almost unknown 20 years ago, involves not one but several different proteins, some of which elicit high-affinity IgE antibodies and others only low-affinity antibodies. This diversity in the affinity of the IgE response is addressed in the study of Handlogten *et al.*¹. Using a

defined synthetic multivalent 'allergen' with both high- and low-affinity binding sites for IgE, they show that weak interactions are critical; importantly, they also demonstrate that blocking these interactions can inhibit an allergic reaction both *in vitro* and *in vivo*.

The cells responsible for the rapid and powerful inflammatory reaction known as the immediate hypersensitivity response

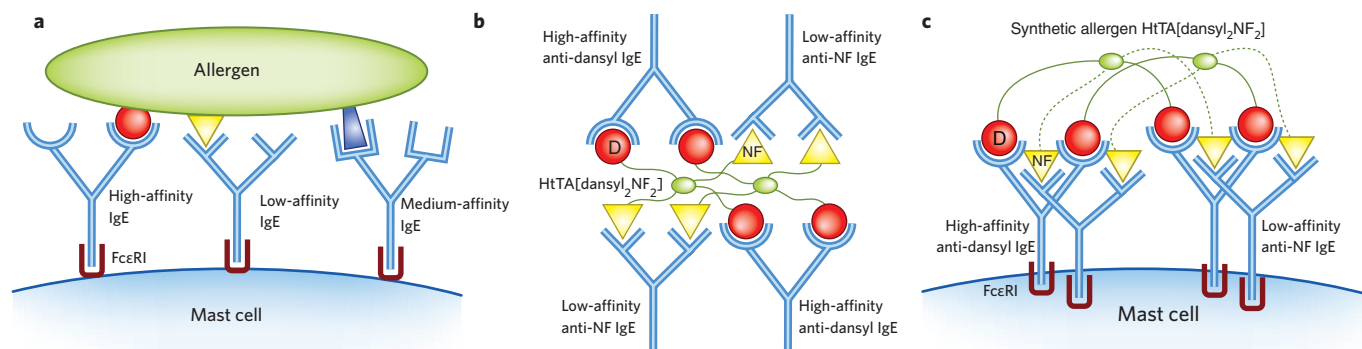


Figure 1 | Multivalent interactions of a protein and a synthetic allergen. **(a)** A protein allergen displaying epitopes of different affinities cross-links specific FcεRI-bound IgE molecules and activates the mast cell. **(b)** Two molecules of the synthetic tetraivalent allergen bearing two dansyl (D) and two nitrofur (NF) moieties, HtTA[dansyl₂NF₂], form a complex with four IgE molecules. The affinity of the NF-IgE interaction is too weak to bind through a single Fab arm, but the avidity resulting from dual-point attachment ensures binding. The synthetic allergen and antibody are not drawn to scale: the separation of the dansyl and NF moieties is designed to be too short to bridge the Fab arms of a single IgE molecule. **(c)** The synthetic allergen HtTA[dansyl₂NF₂] cross-links receptors and activates a mast cell sensitized with both high-affinity anti-dansyl and low-affinity anti-NF IgE antibodies. Inhibition of the low-affinity IgE-NF interactions, indicated by the dashed lines, causes insufficient cross-linking of receptor-bound IgE for cell activation.

are blood basophils and tissue mast cells, the latter residing in the airways of the nose and lung, the skin and the gut, where their activation manifests respectively as hay fever (rhinitis) and allergic asthma, dermatitis and food allergy. The trigger for release of inflammatory mediators such as histamine is cross-linking of their cell surface receptors, which occurs when IgE antibodies already bound to the receptors encounter allergen and are cross-linked. Although IgE is the least abundant of serum antibodies, it binds with such high affinity ($K_d \approx 0.1$ nM) and slow dissociation kinetics ($t_{1/2} \approx 3$ weeks) to the receptor FcεRI that mast cells are primed for immediate response to allergen². Allergens display a range of surface features called epitopes that are recognized by IgE, some strongly, some weakly, and so-called 'immunodominant' epitopes are recognized by more IgEs than others. Cross-linking of receptor-bound IgE requires the allergen to be multivalent, with either more than one copy of a given epitope (if multimeric) or different epitopes to which IgE antibodies of different specificities will bind (Fig. 1a). There may also be steric requirements for the disposition of the epitopes in order to bridge receptor-bound IgE³. Mast cell activation thus involves a complex interplay of valency, affinity (and dissociation kinetics), epitope arrangement and concentration of the various IgE specificities. Addressing this complexity is a challenge.

Earlier studies employed single hapten species presented in various multivalent contexts to investigate valency and epitope spacing and concluded that whereas dimers may sometimes trigger cell activation, trimers and higher multimers are more effective⁴⁻⁷. In the present work, in contrast,

the authors constructed a synthetic allergen that combines two dansyl and two nitrofur (NF) moieties that bind to specific IgEs with high ($K_d = 54$ nM) and low ($K_d = 41$ μM) affinity, respectively, with no cross-reactivity. This more realistically mimicked a protein allergen with its combination of low- and high-affinity epitopes. The haptens were separated by synthetic spacers of a length too short to span the two antigen-binding Fab arms of the same antibody, so that four IgEs might be expected to bind to the heterotetraivalent allergen, termed HtTA[dansyl₂NF₂]. However, the affinity of the IgE specific for NF was too low for single Fab arm attachment, and the authors showed, by determining the hydrodynamic radius using dynamic light scattering, that a single species forms consisting of two HtTA[dansyl₂NF₂] molecules linked by two anti-dansyl and two anti-NP IgE antibodies (Fig. 1b). The avidity effect of two-point attachment enabled the low-affinity anti-NF antibody to join the complex. Could this synthetic allergen similarly cross-link IgE molecules on the surface of a mast cell and cause activation?

Using the well-characterized rat basophilic leukemia cell line, the authors sensitized the cells with equimolar amounts of anti-dansyl and anti-NF antibodies and found that HtTA[dansyl₂NF₂] did trigger activation of the cells. A homotetraivalent allergen, HmTA[dansyl₄], similarly activated the cells, as expected, but HmTA[NF₄] did not, and neither did a homobivalent allergen, HmBA[dansyl₂]. These studies suggest that the low-affinity interactions alone are not sufficient to activate mast cells, and neither is bivalent high-affinity cross-linking, but low-affinity epitopes act

synergistically with high-affinity epitopes to trigger activation. Low-affinity interactions are easier to inhibit, and the authors went on to show that a molecule containing the NF moiety (linked to an indole derivative that targets a conserved hydrophobic site between the variable domains of the antibody^{8,9} to enhance its avidity), inhibited cell degranulation when administered together with HtTA[dansyl₂NF₂]. Removal of the weak interactions led to insufficient receptor cross-linking for cell activation (Fig. 1c). They further demonstrated a dose-dependent inhibition with the same NF-indole molecule in a mouse model of passive cutaneous anaphylaxis, in which the mouse is sensitized with anti-dansyl and anti-NF antibodies and then challenged with the HtTA[dansyl₂NF₂] allergen.

Reducing the overall valency of an allergen by targeting low-affinity IgE-epitope interactions thus appears to be a viable therapeutic strategy, and the synthetic allergen approach with epitopes of known affinity and valency elegantly demonstrates the contribution of the weak component. This is a major step toward a more realistic model of protein allergens, but the reality is undoubtedly more complex still, with numerous different low-affinity epitopes on one protein. Furthermore, a protein epitope may be recognized in different ways by different antibodies. The complexity of the human response to a major house dust mite allergen has been explored by Lund and colleagues, who showed that the diversity of different antibodies, their affinity and their specificity all contribute to determine whether cell activation, and by extension clinical allergy symptoms, result¹⁰. Intervention by a single inhibitor is unlikely

to be effective. Nevertheless, the synthetic allergen approach is amenable to modeling additional complexity, with haptens replaced by peptide epitope mimics and more than one low-affinity antibody. Achilles had just one weak spot that led to his downfall; an allergic response might prove to be more resilient in response to an attack at a single point. ■

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ANTIGENIC PEPTIDES

Reviving nuclear translation

Noncanonical translation of prespliced mRNA provides physiological meaning to nuclear translation in generating antigenic peptide substrates for the endogenous major histocompatibility complex class I pathway.

Petra Van Damme & Gerben Menschaert

In 1974, the cell surface presentation of antigenic peptide-bound major histocompatibility complex class I molecules (pMHC class I) was found to mediate CD8⁺ T-cell immunity. It was initially believed that antigenic peptides were raised by proteasomal protein degradation. Recent evidence, however, has suggested that unconventional sources of peptide epitopes generated by a distinct mRNA translation process contribute to the MHC I ligandome^{1,2}. For example, mRNA-derived full-length protein production was shown to lag far behind production of antigenic peptides¹, pointing to pioneer translation products generated during early stages of mRNA maturation or otherwise defective ribosomal products recently shown to include translation products from alternative or defective mRNAs (that is, cryptic epitopes originating for alternate reading frames, introns, nontranslated regions, downstream initiation and stop codon readthrough) as providing alternative sources of biological relevant antigens for MHC class I molecules^{1–3}. These studies have reoriented the focus toward protein synthesis rather than protein degradation as the source of antigenic peptide substrates. New results from Apcher *et al.*⁴ now infer a spatiotemporal dimension to MHC class I antigenic peptide production in which substrates are produced within the nucleus before mRNA matures (Fig. 1).

The concept of nuclear translation, described for the first time by Allfrey *et al.*⁵ in the early 1950s, has been heavily debated, though with few converts in the intervening years. Despite some favorable evidence for

nuclear translation, the lack of controls and use of impure nuclei or damaged cells meant that this evidence had little influence on the prevailing view of the cytoplasm being the scene of restricted translation. Recently, however, the concept of nuclear translation was revived in a study by David *et al.*⁶, which demonstrated substantial nucleolar enriched translation in intact cells by means of ribopuromycylation, creating stably associated complexes of ribosomes with puromycylated nascent chains. Interestingly, this study also showed that nuclear translation was specifically inhibited upon influenza infection. One explanation for this result is that nuclear translation functions in the quality control of mRNA transcripts by processes such as nonsense-mediated decay (NMD), a surveillance pathway that reduces errors in gene expression by eliminating mRNA transcripts containing premature stop codons (PTCs), which generate defective ribosomal products as a consequence. Another attractive hypothesis is that certain viruses have evolved mechanisms to prevent antigenic peptide formation during the pioneer round of mRNA translation¹.

Apcher *et al.*⁴ address this controversial topic from a different angle by tracking the translational origin of MHC class I peptides. Employing the high sensitivity of detection of MHC class I-presented peptides by specific T cells, the authors introduced antigenic peptide-encoding sequences (or antigenic epitopes), known to be presented on specific MHC class I molecules, in the exonic or intronic sequences of β -globin, with or without

a PTC to provoke NMD. Irrespective of the identity of the antigenic peptide, the intronic or exonic location of its mRNA or whether the mRNA was targeted for the NMD pathway, MHC class I-specific peptide presentation could be observed in all cases where the epitope was in frame with the main open reading frame (ORF). This combined data lead to the conclusion that ribosomal activity on prespliced mRNA produces pioneering translation products and that these are an important source of antigenic peptide substrates for the MHC class I pathway. The authors further provide evidence that epitope expression correlates with nuclear retention of its spliced or unspliced mRNA origin, as nuclear mRNA export decreased whereas nuclear retention increased antigen presentation. Finally, ribopuromycylation and proximal ligation assays demonstrated the presence of nascent intron-derived antigenic peptides in close proximity with ribosomal proteins within the nuclear compartment. Overall, these data elegantly demonstrate a role for nuclear translation in generating antigenic peptides from constructs while providing a logical explanation for the need for compartmentalization in limiting competition for MHC I accessibility.

The results from Apcher *et al.*⁴ will definitely revive interest in nuclear translation by adding translation products from prespliced mRNAs to the list of antigenic peptide sources. However, one caveat to this work is that cellular stress or non-natural situations—such as the overexpression system used—might alter