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Speciation via Autoimmunity: A Dangerous Mix

Nitin Phadnis[1,*] and Harmit S. Malik[2,3,*]
[1]Department of Biology, University of Utah, Salt Lake City, UT 84112, USA
[2]Division of Basic Sciences
[3]HHMI
Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA
*Correspondence: nitin.phadnis@utah.edu (N.P.), hsmalik@fhcrc.org (H.S.M.)
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In this issue of *Cell*, Chae et al. find that genomic "hot spots" encoding NLR plant immune receptor genes are recurrently responsible for hybrid necrosis, highlighting the role of host-pathogen evolutionary arms races in driving the evolution of hybrid incompatibilities.

The evolution of new species involves the establishment of reproductive isolating mechanisms such as hybrid sterility or hybrid inviability between previously interbreeding populations (Coyne and Orr, 2004). Understanding the molecular basis of hybrid incompatibilities—the deleterious genetic interactions that are responsible for hybrid defects—is a profound problem in biology. Two questions are of particular interest in this regard. First, are particular genes or molecular pathways prone to playing a recurrent role in the manifestation of hybrid incompatibilities? Second, what are the biological forces that drive the emergence and spread of incompatible alleles in populations?

Studying hybrid incompatibilities within species provides a powerful approach to study speciation; the same hybrid incompatibilities that segregate within species may also provide the raw material for the establishment of reproductive isolation between species. For instance, hybrid necrosis is a commonly observed defect in many inter- and intraspecific crosses in plants. In this issue of *Cell*, Chae et al. provide a detailed genetic analysis of hybrid necrosis between strains of *Arabidopsis thaliana* collected from diverse geographical locations (Chae et al., 2014). The scale of their analysis is staggering. Through thousands of crosses involving 80 completely sequenced strains of *A. thaliana* (Cao et al., 2011), the authors identify 142 cases of F1 hybrid necrosis. Of these, seven were picked for further genetic analyses in which the causal allele was likely to be present in multiple genetic backgrounds, as evidenced by the similarity of F1 hybrid phenotypes produced in crosses of one parent with several other parental backgrounds. An analysis of F2 offspring using genotyping by sequencing identified seven new hybrid necrosis loci, labeled *DM3* to *DM9* (*DM* stands for *Dangerous Mix* [Alcázar et al., 2009; Bomblies et al., 2007]). Intriguingly, nearly all of the *DM* loci encode plant immune NLR (nucleotide-binding domain and leucine rich) proteins (Spoel and Dong, 2012).

Many of the F1 incompatibilities underlying hybrid necrosis involve pairwise interactions between distinct NLR loci, in accordance with a common portrayal of the Bateson-Dobzhansky-Muller (BDM) model for hybrid incompatibilities (Figure 1A). Under this model, new alleles at separate loci can arise and become fixed in populations because they are compatible with the genetic backgrounds in which they arose. However, these new alleles cause problems in hybrids when they do not function properly together. Surprisingly, *DM8* and *DM9* involve deleterious heterozygote interactions at the same genetic locus. Such single locus hybrid incompatibilities are rare and have generally been thought to be unlikely because a new incompatible allele that is sufficient to cause hybrid dysfunction must necessarily originate in a heterozygous state and therefore be instantaneously deleterious. Examples include speciation between dextral and sinistral versions of snails (Orr, 1991; Ueshima and Asami, 2003). Another way out of this conundrum is the sequential fixation of new alleles at the same locus, as appears to be the case with the *DM8*- *and DM9*-incompatible alleles (Figure 1B).

The identification of the incompatible NLR alleles of *DM8* and *DM9* also suggests a specific biochemical possibility to explain still poorly understood aspects of NLR protein activation. For instance, pioneering work studying mechanisms of plant NLR protein activation has revealed that they can be activated either directly by specific pathogen effectors ("non-self") or by effector-mediated modifications of other "signaling hub" host proteins ("modified self") such as the RPM1-INTERACTING PROTEIN 4 (RIN4) (Maekawa et al., 2011; Spoel and Dong, 2012) (Figure 1C). Yet, what molecularly activates plant NLR proteins is still somewhat mysterious. The fact that *DM* alleles can cause hybrid necrosis with such high penetrance must imply that this combination of NLR proteins leads to NLR activation. This could be because each NLR protein is incompatible with a variant of a modified self protein (e.g., RIM4) from the other genome, resulting in cross-activation (Spoel and Dong, 2012). However, if this were the case, the other locus should be genetically identifiable as a hybrid incompatibility locus. Alternatively, the direct interaction of these incompatible NLR proteins with each other might directly activate the host necrosis response. Under this scenario, an NLR protein might activate by recognizing an incompatible NLR partner as a modified self protein (Figure 1D). In this regard, it is probably not a coincidence that Chae et al. find the *DM2* locus to be involved

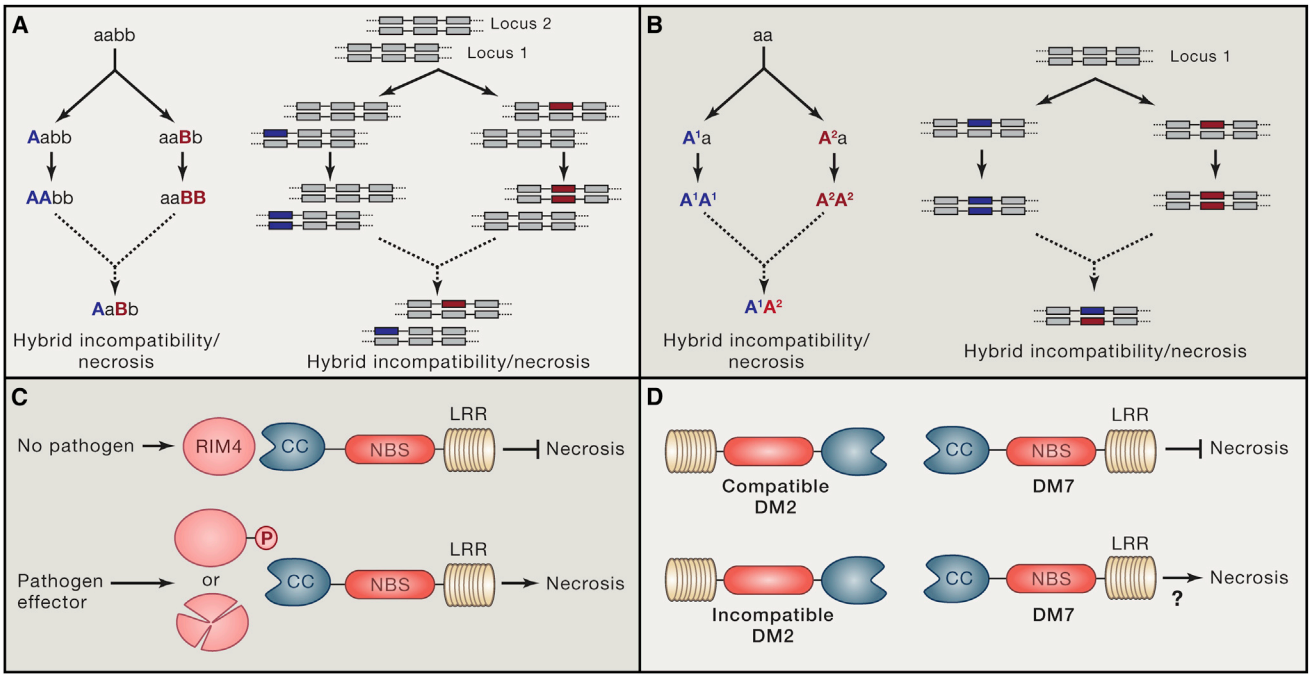

Figure 1. Hybrid Incompatibilities in NLR Genes Lead to Necrosis in *Arabidopsis*

(A) Consistent with the two-locus version of the Bateson-Dobzhansky-Muller (BDM) model for the evolution of hybrid incompatibilities, Chae et al. show that incompatible alleles could arise on different NLR clusters to cause incompatibility and hybrid necrosis.

(B) *DM8* and *DM9* represent NLR alleles that each arose in isolation with compatible ancestral DM versions but become incompatible in the novel, untested configuration, consistent with the much rarer single-gene model of hybrid incompatibility.

(C) Plant NLR genes frequently activate to trigger immunity (e.g., necrosis) upon sensing "self" proteins that have been modified by pathogen effectors i.e., "modified self" proteins. For instance, pathogen effector-mediated phosphorylation or fragmentation of the host protein RIM4 leads to specific activation of certain NLRs.

(D) We speculate that the incompatibility between NLR proteins might arise because allelic differences in the *DM2* locus (compatible versus incompatible alleles) are interpreted as modified self proteins, triggering NLR activation and therefore hybrid necrosis.

in at least five out of nine known cases of F1 hybrid necrosis (Chae et al., 2014). *DM2* could represent an example of a signaling hub protein in which incompatible NLR proteins interpret allelic differences as modified self instead. *DM2* may be especially susceptible to triggering hybrid necrosis because its enzymatic activity may be directly coupled to downstream signaling events that induce cell death. Study of the biochemical differences between the hybrid necrosis risk and nonrisk alleles of NLR genes is likely to reveal insights into what activates NLR proteins and what keeps them in check.

Hybrid incompatibilities cause severely deleterious fitness consequences; the incompatible alleles are certainly not selected for these properties. Indeed, one might expect alleles with a propensity to be incompatible to have a significant selective cost within populations. Instead, hybrid incompatibilities are likely an accidental consequence of the evolution of these genes for other reasons. Biological phenomena that drive the rapid and recurrent evolution of genes, such as intragenomic conflict (Phadnis and Orr, 2009) or host-pathogen arms races, provide strong candidates for the engine of speciation. The increased genetic repertoire of NLR genes in plants (150 in *Arabidopsis* and 450 in rice) as compared to vertebrates (~20) (Maekawa et al., 2011), together with their rapid evolution, might simply increase the odds of incompatible combinations, or "a dangerous mix." This might explain why hybrid incompatibility due to NLR genes is so rampant in plants.

Crop breeders sometimes actively select hybrid necrosis genes to derive strains that are resistant to particular pathogens. It is almost certain that natural selection has done this for far longer, in a "tit-for-tat" between plant NLR proteins and plant pathogen effectors. Although this rapid adaption may confer pathogen resistance, Chae et al. find that such immunity might recurrently levy an accidental but high cost in occasionally producing incompatible combinations that cause autoimmunity and hybrid necrosis (Chae et al., 2014), sowing the seeds of speciation.

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A Split Personality for Nucleosomes

Daniel J. McKay[1] and Jason D. Lieb[2,*]

[1]Department of Biology, Department of Genetics, Integrative Program for Biological and Genome Sciences, The University of North Carolina at Chapel Hill, 250 Bell Tower Drive, Chapel Hill, NC 27599, USA

[2]Department of Human Genetics, The University of Chicago, 920 East 58th Street, Chicago, IL, 60637, USA

*Correspondence: jdlieb@uchicago.edu

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A high-resolution look at where histones touch DNA reveals a surprisingly intricate, dynamic, and modular nucleosome. Three advances in the study by Rhee et al. include unexpected interactions between the H3 tail and linker DNA, new evidence for existence of subnucleosomal particles, and asymmetric patterns of histone modification within a single nucleosome that correspond to the direction of transcription.

In eukaryotic cells, DNA is packaged into nucleosomes. A single nucleosome consists of a protein spool made of histones, wrapped by DNA. In addition to packaging DNA, nucleosomes also compete with other DNA-binding proteins and thereby influence access to the regulatory information that controls DNA-dependent processes such as transcription, replication, and DNA repair. In this issue of *Cell*, Pugh and colleagues (Rhee et al., 2014) apply a high-resolution mapping approach called ChIP-exo in yeast to examine the genome-wide position and organization of the individual histones that comprise nucleosomes. Their findings reveal surprisingly complex nucleosome substructures and dynamics that immediately bring to light an exciting set of new questions for the field, while at the same time evoking early models of the nucleosome (Weintraub et al., 1976).

Some background is required to set the stage for the three major advances derived from the results. The traditionally defined nucleosome core consists of an octamer of histone proteins, around which ~147 bp of DNA is wrapped. This octamer is composed of two copies each of the histones H2A, H2B, H3, and H4. More specifically, dimers of H3 and H4 interact to form a tetramer, which is flanked on each side by a dimer of H2A and H2B. Pugh and colleagues used ChIP-exo to determine the precise location of individual histone proteins across the yeast genome. ChIP-exo is a modified version of conventional chromatin immunoprecipitation (ChIP) that provides high-resolution identification of binding sites for proteins that interact with DNA. Like ChIP, the first step in ChIP-exo is to covalently crosslink proteins to DNA with formaldehyde. After sonication to shear the chromatin into smaller fragments and immunoprecipitation with antibodies that recognize the protein of interest, ChIP-exo then uses lambda exonuclease to digest DNA strands in the 5′ to 3′ direction. Digestion is blocked when the exonuclease reaches a protein-DNA crosslink. After high-throughput sequencing, pairs of 5′ ends on the forward and reverse strands (exonuclease stop points) thus represent the boundaries of a given protein-DNA interaction. ChIP-exo has previously been used to map binding sites for sequence-specific transcription factors (Rhee and Pugh, 2011), preinitiation complexes (Rhee and Pugh, 2012), and chromatin remodelers (Yen et al., 2012; Yen et al., 2013).

The first intriguing result of Rhee et al. (2014) concerns the amino-terminal tail of histone H3, which is heavily decorated with posttranslational modifications and has important regulatory functions. ChIP-exo results for H2B and H4 histones identified crosslinking points that closely correspond to the genomic locations expected from the crystal structure (Luger et al., 1997). On the other hand, ChIP-exo results for histone H3 showed an unexpected crosslinking pattern. In the crystal structure, most of the amino acids comprising H3 reside at the nucleosome midpoint (called the "dyad"), where they contribute substantially to DNA interactions (Luger et al., 1997). However, the predominant H3-DNA interaction determined by ChIP-exo was located in the linker DNA that separates adjacent nucleosomes, not at the nucleosme dyad. The authors speculated that this interaction may be mediated through the N-terminal tail of histone H3, and then tested their