activating receptor KIR2DS2 to HLA-Cw*07 shown in a *bona fide* binding assay[13]. In that regard, the work of Desrosiers *et al.* justifies creativity in considering ligand mates for activating KIR molecules. The specific Ly49P recognition of MCMV-infected H-2D^k targets and not cells infected by a second herpesvirus (MHV68) also suggests potential restriction in ligand specificity of other activating KIR or Ly49 molecules. Although it makes good sense that the finite number of KIR or Ly49 activating receptors should be designed to tackle many different pathogens, including newly emerging ones, it also seems reasonable that specificity of certain activating receptors for a particular pathogen might evolve if that pathogen has been a persistent foe throughout the species' history.

Timely triggering

One of the most difficult tasks delegated to the immune system is to be prepared to handle infectious organisms with sufficient force to protect the host but to remain controlled enough to avoid self-destruction. Whereas inhibitory signals dominate over activating signals during hale and hearty times, the scale must tip towards activation upon encounter with an infectious assailant to an extent appropriate given the pathogenicity of the invading organism. Desrosiers *et al.* note that even though the resistant MA/My mice express on some NK cells an inhibitory receptor, Ly49I, which binds to the MCMV m157 protein (*i.e.*, the same ligand recognized by the activating receptor Ly49H), this signal does not prevent Ly49P–H-2D^k–mediated activation of MA/My NK cells against MCMV. Apparently, neither does the inhibitory molecule Ly49V which, like Ly49P, recognizes H-2D^k (ref. 14) and is encoded on the MA/My *Klra5*^{m} haplotype.

Some viruses, including MCMV, reduce MHC class I expression to escape immune recognition by cytotoxic T lymphocytes[15], but they don't generally shut down the works completely. Accordingly, Ly49P still seems able to detect H2 ligands on infected targets efficiently enough to control viral pathogenesis. This observation raises an issue related to the balance between NK cell inhibition and activation: does low class I expression somehow shift its attraction as a ligand from inhibitory to activating Ly49 or KIR receptors? It is notable that HLA-C molecules, the primary ligands for KIR receptors in humans, are generally poorly expressed relative to HLA-A or HLA-B, which could represent a possible safeguard against too much inhibition when viral infections strike.

The work of Desrosiers *et al.* leaves us with many small and large questions. Will *in vitro* killing assays support the *in vivo* resistance that we assume is due to efficient Ly49–H-2D^k–mediated elimination of infected targets? Can susceptible mice that have *H2*^{k}, but not *Klra16*, become resistant following introduction of a *Klra16* transgene? Will some of the genetic complexity of the resistance trait seen in F_2 progeny lead to identification of additional genetic modifiers? What is the nature of the MCMV specificity of Ly49P activity? Does it involve presentation of a MCMV-derived peptide or downregulation of ligands for the inhibitory Ly49I or Ly49V molecules? Are there additional classes of ligands for activating Ly49 besides viral proteins and host H2, such as self proteins generated upon the stressful circumstance of viral infection? How much will the new findings about activating Ly49 receptors in mice hold true for the activating KIR molecules in humans? And there are more, no doubt. Good research answers a question or two, but perhaps more importantly, it raises further questions that are succinct, informed and worth pursuing.

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The gene duplication that greased society's wheels

Meg Woolfit & Ken Wolfe

Yeast's ability to produce ethanol in high concentrations has been exploited by humans for millennia. Two recent papers help us to understand the genetic changes that made this species so appealing to humans and the history of its domestication.

Imagine a world without alcohol. No pinot noir, no Guinness, no single malt whisky…no home-made parsnip wine at grandma's, to look on the bright side. All it would take for this scenario to become reality is the loss of a single species of yeast, *Saccharomyces cerevisiae*. This yeast, with its superlative fermenting ability, is responsible for almost all the alcohol produced by humanity, from traditional African palm wine to the most technologically finessed Californian cabernet. How did this species come to occupy such a vital role in human affairs? Two new papers[1,2] discuss the evolution of yeast's ethanol metabolism and how humans have harnessed it for their benefit.

Many microorganisms are capable of fermenting sugars to obtain energy, synthesizing ethanol as a by-product. This metabolic pathway is limited, however, by the toxicity of ethanol, which destabilizes cellular membranes and disrupts other aspects of metabolism[3]. *S. cerevisiae* possesses numerous adaptations that allow it to generate, and tolerate, external concentrations of ethanol that are so high (7–9% in typical fermentations[4]) that they are lethal to most of its microbial competitors. One such adaptation is yeast's ability to first make ethanol (by fermentation, which occurs even in aerobic conditions) and later consume it (by respiration). This is achieved by the differential regulation of two duplicated alcohol dehy-

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drogenase genes, *ADH1* and *ADH2*. The Adh1 isoform is constitutively expressed and reduces acetaldehyde, a product of the breakdown of glucose, to ethanol. Adh2, which oxidizes ethanol back to acetaldehyde, becomes derepressed as glucose levels decrease, allowing yeast to begin living off the ethanol it has made.

Old wine in new bottles

Enjoyable as its results are for us, the evolution of this behavior presents a biochemical conundrum. It is energetically wasteful, and often biochemically unnecessary, to metabolize glucose through an ethanol intermediate. On page 630 Thomson *et al.*[1] report on their use of a combination of molecular sequence analysis and genetic manipulation to tease out an evolutionary explanation for this seeming inefficiency by addressing two key questions: whether the ancestral yeast, before the duplication of *ADH1* and *ADH2*, was adapted for production or consumption of ethanol, and what selective pressure may have led to the development of this extended metabolic pathway.

To determine the function of the original single *ADH* gene, Thomson *et al.* took a hands-on approach. They used molecular phylogenetic methods to infer 12 possible sequences of the ancestral gene and then synthesized these variants and transformed them into a strain of *S. cerevisiae* from which both *ADH1* and *ADH2* had been deleted. Each reconstructed gene was functional, and all but one showed greater catalytic activity from acetaldehyde to ethanol than in the opposite direction. It therefore seems likely that the ancestral yeast species was able to ferment sugars into ethanol but could not consume ethanol as efficiently as modern yeast does. This secondary function was probably gained only after the ancestral gene was duplicated.

Drunken dinosaurs?

Duplicated genes are likely to be lost from the genome unless natural selection acts to preserve both copies[5]. Once the ancestral *ADH* gene had been duplicated, what selective pressure led to the retention of the second copy and the subsequent development of the ability to metabolize ethanol? Thomson *et al.* suggest two possibilities: first, that it gives yeast an advantage against less ethanol-tolerant competitors in its natural environment of decaying fruit[3,6], and second, that the emergence of increased ethanol tolerance may have been driven by humans selecting for yeasts that produced greater quantities of alcohol. Two lines of evidence suggest that the former explanation is correct and that humans have simply taken advantage of a process that yeast itself has been exploiting for its own benefit for millions of years.

Bottom panel of the 4,500–4,600 year old "Great Lyre from the King's Grave" the Royal Cemetery of Ur. The scene depicts a gazelle offering two beakers of beer to a scorpion man. Photo courtesy of the University of Pennsylvania Museum of Archaeology and Anthropology (neg. #T4-887).

The first line of evidence relies on molecular clock analysis, which suggests that the duplication of the ancestral *ADH* gene occurred shortly after the divergence of the *S. cerevisiae* and *Kluyveromyces lactis* lineages[1], which has been dated to ~80 million years ago[7]. This is consistent with the estimated time of origin of fleshy fruits during the Cretaceous period[8], rather than the much more recent origin of human-controlled alcohol production, the earliest evidence for which comes from 9,000 years ago (ref. 9). The gene duplication that produced *ADH1* and *ADH2* happened somewhat more recently than the whole-genome duplication that occurred in an ancestor of yeast[10]. In fact, Thomson *et al.* identify several gene pairs in yeast, all involved in ethanol production or consumption, all estimated by a molecular dating method to have duplicated since the whole-genome duplication. This is remarkable given the relatively low number of recent gene duplications in the yeast genome and suggests that strong selective pressure has favored yeast's strategy of making life difficult for other microorganisms by rapidly converting the available sugars into ethanol, which is later respired.

Further evidence that humans merely domesticated yeast after it had already established this strategy comes from Fay and Benavides[2]. They sequenced five loci from a wide range of *S. cerevisiae* strains taken from fermentations, tree exudates and human individuals. Phylogenetic analysis of these sequences showed that the earliest branches on the tree are from natural fermentations and clinical isolates, whereas wine and saké strains of yeast form two separate clades branching late in the tree, each with low genetic diversity. This suggests that *S. cerevisiae* was domesticated on at least two independent occasions from a diverse wild population that already possessed the ability to produce high concentrations of ethanol.

Alcohol has been used by humans for millennia and has a central role in civil and religious ceremonies in many human cultures. Its impact on civilization has been immense. As Homer Simpson once put it, alcohol is the cause of, and the solution to, all of life's problems. This makes us wonder what life would be like if the gene duplication that formed *ADH2* hadn't happened. Would we live in a teetotal society, or would our ancestors have used another ethanol-producing microorganism (perhaps *Brettanomyces*, as used in 'lambic' beers[11]) instead? Something to ponder while you wait for your pint to settle.

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