eventually join two DSB ends together. The studies by Alt, de Villartay, and Roth and their colleagues pave the way for determining the genetic requirements for these alternative DNA repair pathways and for establishing their role in the chromosomal translocations that cause cancer.

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When Two Is Better Than One

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DOI 10.1016/j.cell.2007.10.001

Gene duplication and divergence has long been considered an important route to adaptation and phenotypic evolution. Reporting in *Nature*, Hittinger and Carroll (2007) provide the first clear example of adaptations in both regulatory regions and protein-coding regions after gene duplication. This combination of evolutionary changes appears to have resolved an adaptive conflict, leading to increased organismal fitness.

Genes that perform more than one function can get caught in an evolutionary tug of war, when improving one function necessarily compromises another. Gene and genome duplications have long been considered an important means of breaking these adaptive deadlocks. Original models of gene duplication were developed under the neutral theory: following duplication, one gene would be constrained to perform the ancestral function, whereas the other, its paralog, would be free of selective constraints and therefore able to take on a new function (Ohno, 1970). Decades later, it is clear that many genes perform more than one function prior to duplication, often through alternative regulatory inputs, splicing, or post-translational processing. More recent models have focused on these processes as an explanation for the survival of duplicated genes (Hughes, 1994; Hughes, 1999). The duplication, degeneration, and complementation (DDC) model, for instance, posits that paralogs that perform some subset of the ancestral functions can be preserved through complementary loss of regulatory sequences without invoking natural selection (Force et al., 1999). One example of this is in the Hoxb1 paralogs of zebrafish, Hoxb1a and Hoxb1b, which appear to have developed subfunctions after the whole-genome duplication within the bony fishes. Hoxb1a and Hoxb1b expression domains have become subdivided among paralogs due to complementary degenerative mutations in *cis*-regulatory sites, whereas most protein function appears to be largely conserved following duplication (McClintock et al., 2002). This regulatory divergence is consistent with a DDC-like process that occurred in the absence of adaptation. However, the relative roles of selection and degeneration, whether selection works on mutations in regulatory or

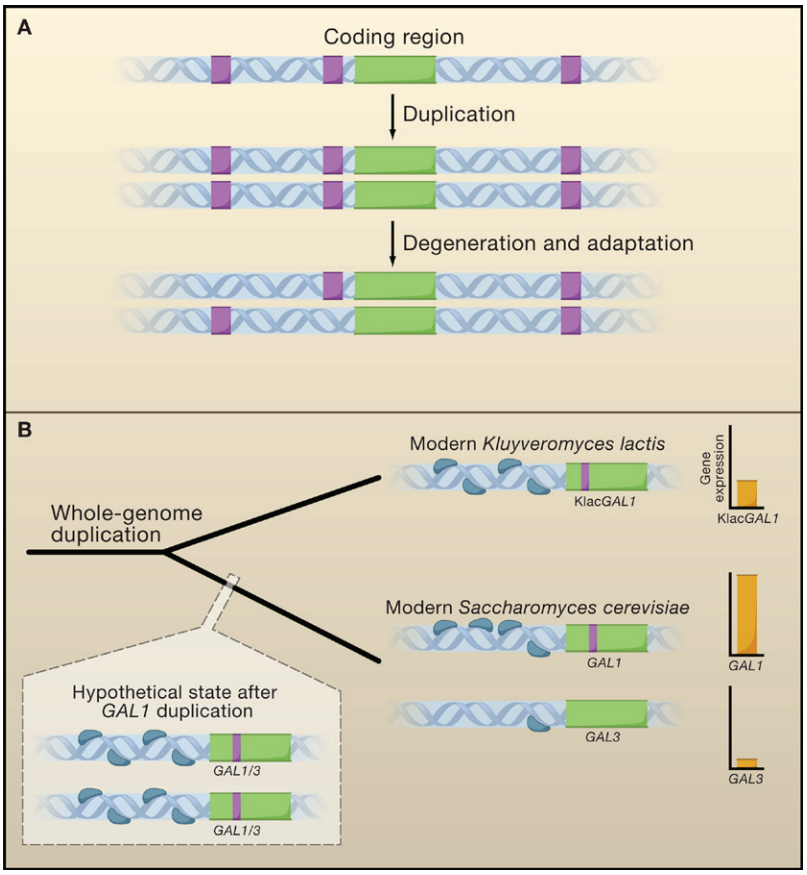

Figure 1. Resolution of an Adaptive Conflict

(A) Before duplication, interactions between regulatory regions (purple) can constrain changes in sequence and function. After gene duplication, complementary degenerate mutations can occur, either in regulatory regions as shown here or within the coding region (green) of the gene (as in the loss of the SA dipeptide in panel B). As each of the duplicates assumes some of the subfunctions of the ancestral gene, they will be maintained as functional duplicates, with some sites conserved. Yet, the duplicates are less constrained than the ancestral gene, and some sites can degenerate, presumably due to decreased pleiotropy. Selection can then act on the paralogs independently, allowing for adaptation of gene expression or function.

(B) Model of the evolution of the *S. cerevisiae GAL1* and *GAL3* paralogs. *K. lactis* has not undergone a whole-genome duplication, and the galatokinase and coinducer functions are encoded by one locus, Klac*GAL1* (top). After whole-genome duplication the regulatory regions of the paralogs in the *Sacchraromyces* lineage subfunctionalize as in the DDC model. The evolution of the *cis* phasing (binding to the same side of the DNA double helix) of the Gal4p binding sites in *GAL1* and the loss of some of those sites in *GAL3* occurred before the diversification of species within *Saccharomyces sensu stricto* (the group containing *S. cerevisiae* and its closest relatives), whereas the loss of the SA dipeptide (purple bar) and the associated galactokinase function of *GAL3* occurred after that diversification (bottom). The qualitative effects of these changes on gene expression are illustrated to the right (orange bars).

coding sites, and how this becomes a route for adaptation, remain open questions.

In their new study, Hittinger and Carroll (2007) report the first specific evidence of the genetic changes involved in the resolution of an adaptive conflict through gene duplication (Figure 1A). Their study reveals elements of both models of gene duplication, through a detailed dissection of changes in both regulatory regions and protein-coding regions after gene duplication in the well-understood galactose metabolic pathway of the budding yeast *Saccharomyces cerevisiae*. The *S. cerevisiae* genes *GAL1* and *GAL3* are paralogs presumably created during a whole-genome duplication event, which occurred around 100 million years ago (Kellis et al., 2004). The functional evolution of these paralogs was explored by Hittinger and Carroll using an outgroup species of yeast that did not undergo a whole-genome duplication, *Kluyveromyces lactis*. The *KlacGAL1* gene of this yeast performs the functions of *GAL1* and *GAL3* in *S. cerevisiae*. *GAL1* and *GAL3* belong to a well-known network of genes in yeast whose products metabolise galactose in the absence of glucose. *GAL1* encodes the galactokinase Gal1p, and *GAL3* encodes the coinducer Gal3p. Gal3p sequesters the *GAL1* repressor Gal80p, allowing for *GAL1* activation by Gal4p (Platt and Reece, 1998). The pathway is similar in *K. lactis* except that *KlacGAL1* encodes a single protein, KlacGal1p, that performs both the galactokinase and coinducer functions (Rubio-Texeira, 2005).

Hittinger and Carroll explored the consequences of this specific gene duplication in both regulatory regions and protein-coding regions using sensitive competitive growth assays. Although both Gal1p and Gal3p can act as the coinducer in this pathway, in *S. cerevisiae* only Gal1p functions as a galactokinase. The essential functional changes in the coding region are thought to hinge on a serine-alanine (SA) dipeptide, which confers some galactokinase function to Gal3p if inserted into the active site. However, in competitive growth assays, Gal3p with the SA dipeptide shows fitness defects due to a slower enzymatic rate relative to Gal1p when galactose is present. Wild-type KlacGal1p also shows a fitness defect, but this appears to be due to toxicity of KlacGal1p overexpression in *S. cerevisiae*. The authors tested whether this fitness defect of Gal3p with the SA dipeptide is due to an adaptive conflict due to the SA dipeptide and found that this apparently was not the case. Instead, removal of the SA residues from Gal1p and KlacGal1p reduced their coinduction function relative to the wild-type state. The authors conclude that this is a case of "sign epistasis," a situation where the fitness effect is dependent on the context of other changes in other sites in the protein. A mutation that increases fitness for one protein can decrease it in another, similar protein.

The authors also investigated regulatory changes, which appear to be the more ancient set of changes after gene duplication. These changes seem

to have involved subfunctionalization and later optimization in the regulatory regions of *GAL1* and *GAL3* in the lineage leading to *S. cerevisiae*. They found that replacing Gal3p with Gal1p resulted in decreased fitness relative to wild-type under inducing conditions. In addition, this strain does much better than a strain missing not only the *GAL3* coding sequence but also the *GAL3* promoter. Taken together, this suggests that *GAL1* and *GAL3* have also undergone subfunctionalization of their promoters, P_{GAL1} and P_{GAL3}. As a result of these changes, neither promoter is now a satisfactory substitute for the other. Expression of Gal1p from P_{GAL3} under inducing conditions is too low to permit efficient metabolism, and basal expression of Gal3p from P_{GAL1} is too low to permit efficient induction. $P_{KlacGAL1}$ is also inferior to the modern *S. cerevisiae* P_{GAL1}. Expression of Gal1p from $P_{KlacGAL1}$ under inducing conditions is about 50% that from P_{GAL1}, reducing fitness approximately 10%. These results suggest that adaptive mutations have occurred in P_{GAL1}, strengthening its inducibility.

To detail the molecular mechanisms involved in resolving the adaptive conflict, Hittinger and Carroll next tested the connection between fitness and the helical phasing of Gal4p binding sites within the *S. cerevisiae* and *K. lactis GAL1* promoters. The importance of helical spacing of Gal4p binding sites in the activation of *GAL1* has been well documented (Webster and Dickson, 1988). In P_{GAL1}, the three core Gal4p sites are on the same side of the double helix; in $P_{KlacGAL1}$, they are on alternate sides. The authors tested the consequences of evolutionary changes in the binding side (phasing) by modifying $P_{KlacGAL1}$ to the spacing of P_{GAL1} and found that both induced and noninduced expression increases. These results suggest that the adaptive conflict was resolved after duplication by subfunctionalization of the promoter and later optimization of galactose metabolism by changes in the phasing in P_{GAL1} (Figure 1B).

The actual ecological relevance of these mutations remains unclear. Hittinger and Carroll discovered that in *K. lactis*, the modified $P_{KlacGAL1}$ outperforms the wild-type $P_{KlacGAL1}$ at high galactose and lactose concentrations. In this species, limitations to the coinduction function are offset by a benefit to the galactokinase function. Reduced spacing may not be favored under the spectrum of conditions encountered by *K. lactis* in the wild, which could account for the arrangement of Gal4p binding sites in this species during 100 million years of divergence from the common ancestor. Understanding how gene duplication affects fitness under ecologically relevant conditions remains an important next step for this and other studies that examine the evolution of gene function.

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Retinoblastoma Teaches a New Lesson

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DOI 10.1016/j.cell.2007.10.007

In this issue, Ajioka et al. (2007) report a new mouse model of retinoblastoma. They show that retinoblastoma is not driven by uncontrolled expansion of retinal progenitor cells, but rather is the result of cell cycle re-entry and expansion of differentiated horizontal interneurons in the retina.

Retinoblastoma, a rare childhood cancer of the retina, has intrigued scientists for decades. Studies of retinoblastoma have provided pivotal insights into basic mechanisms underlying cancer formation. Retinoblastoma has served as a key model for tumor development caused by loss of tumor suppressor genes since the discovery of the retinoblastoma gene *RB*. The retinoblastoma protein pRB is a central gate-