

EVOLUTIONARY GENETICS

From phenotype to genotype

Phenotypic mutations result from errors in transcription or translation that lead to the production of protein variants from unmodified genes; however, the role of this process in evolution has been unclear. Now, a study on the evolution of protein paralogues provides the first direct evidence that phenotypic mutations paved the way for genetic adaptation through gene duplication.

Yanagida *et al.* focused on the *Saccharomyces cerevisiae* Idp2 and Idp3 proteins, which compartmentalize to the cytosol and the peroxisome, respectively, and diverged following the yeast whole-genome duplication (WGD) event. To investigate the compartmentalization of Idp3, the authors searched for a carboxy-terminal peroxisomal targeting sequence, called PTS1, in *S. cerevisiae*-related species that diverged before or after the WGD. Both pre-WGD and post-WGD species contained an *IDP2* gene encoding a cytosolic isoform, but only post-WGD strains contained an *IDP3* paralogue with a PTS1 signature for peroxisomal targeting. In complementation experiments, only *IDP2* that was modified

to contain PTS1 could complement an *IDP3* mutation. This suggested that, following the WGD, Idp2 remained a cytosolic protein and Idp3 acquired a PTS1 signature, causing it to localize to the peroxisome.

Interestingly, the authors identified putative PTS1 motifs that were +1 nucleotide out of reading frame, downstream of pre-duplication *IDP2* genes in several Saccharomycetaceae species. Unveiling these cryptic motifs through the introduction of a single-nucleotide deletion resulted in peroxisomal targeting of the product, suggesting that transcriptional or translational frameshift errors in an ancestral *IDP2* gene might result in expression of a peroxisomal isoform, in addition to the usual cytosolic isoform. Indeed, mass spectrometry and fluorescent cell imaging confirmed the coexistence of cytosolic and peroxisomal isoforms of Idp2 in a pre-WGD strain containing only an *IDP2* gene with a cryptic PTS1.

Exploring the mechanism of cryptic PTS1 recruitment, the authors examined a homonucleotide repeat situated just upstream of the *IDP2* stop codon of one pre-WGD

strain. The introduction of silent mutations here abolished PTS1 functionality, confirming this as the site of phenotypic mutation. Furthermore, RT-PCR analysis revealed the preservation of this homonucleotide repeat at the RNA level, suggesting that production of peroxisomal Idp protein from ancestral *IDP2* is due to translational, not transcriptional, frameshifting. Remarkably, laboratory evolution experiments indicated that the phenotypic mutations that led to peroxisomal targeting of Idp2 protein became fixed in post-duplication *IDP3* by genetic deletions occurring at the very same site.

These experiments show that *S. cerevisiae* *IDP3* diverged from a pre-duplication *IDP2* gene that already had the capacity for peroxisomal compartmentalization through phenotypic mutation. Therefore, gene duplication did not initiate but rather followed the phenotypic divergence of compartmentalization. These findings suggest that genotypes favouring phenotypic variability enhance the potential for future evolutionary genetic adaptations.

Denise Waldron

ORIGINAL RESEARCH PAPER Yanagida, H. *et al.*
The evolutionary potential of phenotypic mutations. *PLoS Genet.* **11**, e1005445 (2015)

“ These findings suggest that genotypes favouring phenotypic variability enhance the potential for future evolutionary genetic adaptations ”

P. Morgan/NPC