Trends in Biochemical Sciences

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Spotlight

Constructive neutral evolution of homodimer to heterodimer transition

Lin Chou [1,2,3], Carly J. Houghton [1,2,3], Aaron Wacholder [1,2,3], and Anne-Ruxandra Carvunis [1,2,*,@]

Complexification of macromolecules, such as homodimer to heterodimer transitions, are common during evolution. Is such complexification always adaptive? Using large-scale experiments and in-depth biochemical analyses, Després *et al.* recently demonstrated that an obligate heterodimer can evolve from a homodimer through neutral, nonadaptive events, and quantified key parameters required for such transitions.

The relative importance of neutral and adaptive events in evolution has been the subject of a heated debate for half a century [1]. A recent study by Després *et al.* probed the biochemical mechanisms of protein complex evolution with remarkable depth and scale, providing a new way to evaluate the relationship between the two sides [2]. Across evolutionary history, it is common for homodimers to evolve into obligate heterodimers [3]. A theory called 'Constructive Neutral Evolution' (CNE) offers a framework for how this homodimer-to-heterodimer transition can occur even when the heterodimer provides no fitness advantage [3,4]. CNE is a general theory proposed over 20 years ago that formalizes how biological systems can complexify through neutral evolutionary processes [4]. According to this theory, a homodimer can evolve into a heterodimer in a nonadaptive manner following duplication of the gene encoding the homodimer. The duplication gives rise to two copies of the initial gene. Mutations that eliminate the homodimer function in either gene copy can be maintained provided they are compensated for by the second copy. For example, one copy could lose catalytic activity, but still allow a functional heterodimer if the catalytic site is still active in the other copy. If both genes experience loss-of-function (LOF) mutations that are mutually compensated for by the other, then it is no longer possible to lose either copy without losing the function entirely. Thus, the protein pair becomes an obligate heterodimer (Figure 1).

Previous work provided experimental evidence that CNE can explain the emergence of a homodimer from a monomer [5,6] and the diversification of components of a hexamer [7]. However, whether CNE can also explain the homodimer-to-heterodimer transition has largely remained theoretical. For this transition to be favorable, two requirements have to be satisfied: (i) there has to exist a relatively large number of LOF mutations that could be masked in one copy by compensatory mutations in the other copy; and (ii) the number of mutations required for compensation must be few; otherwise, the probability of compensation would be low relative to the probability of simply losing the defective gene [2]. Using a clever experimental strategy, Després *et al.* provide the first estimates of these critical biophysical parameters and demonstrate that CNE can indeed explain homodimer-to-heterodimer transition [2].

Després *et al.* applied a high-throughput forward genetic approach to the homodimeric enzyme cytosine deaminase (Fcy1) in yeast to enumerate the possible mutational paths that favor heterodimer formation following a simulated gene duplication. The systematic step-wise approach involved: (i) selecting LOF mutations in Fcy1 from nearly all possible single amino acid substitutions using deep mutational scanning; then (ii) mating haploid strains with single amino acid LOF Fcy1 variants together to produce a diploid pool, in which each cell has a combination of two LOF Fcy1 variants; and finally (iii) selecting for pairs of LOF variants that rescue Fcy1 function [2]. This strategy allowed the authors to identify and characterize pairs of nonfunctional Fcy1 copies that compensated for each other by forming a heterodimer. Compensatory mutations of Fcy1 copies were not only possible but also frequent, with at least 207 unique heterodimers found to be functional. These heterodimers did not provide a fitness benefit relative to the initial homodimer. Yet, there cannot be a reversion to the homodimer form since each copy has a LOF mutation. Therefore, the authors demonstrate that many potential nonadaptive routes exist for homodimer-to-obligate heterodimer transitions of yeast Fcy1 [2].

How do the mutations in each copy compensate for the LOF effects in the other? Després *et al.* probed this question biochemically with remarkable depth. They found that 93% of the compensatory mutations clustered near the active sites and the dimer interface. Among the compensatory mutation pairs, an active site mutation E64V and a dimer interface mutation M100W were subsequently selected for a follow-up study. Crystallographic data showed that the M100W mutation in one copy impaired the catalytic activity of the opposite copy, rendering the $Fcy1_{M100W}$ homodimer inactive. However, $Fcy1_{M100W}$ and $Fcy1_{E64V}$ formed an active heterodimer because the active site was still active in $Fcy1_{M100W}$. Thus, $Fcy1_{M100W}$ and $Fcy1_{E64V}$ are locked in with each other as a heterodimer: if either copy was lost, the organism would be left with a catalytically dead homodimer (Figure 1).

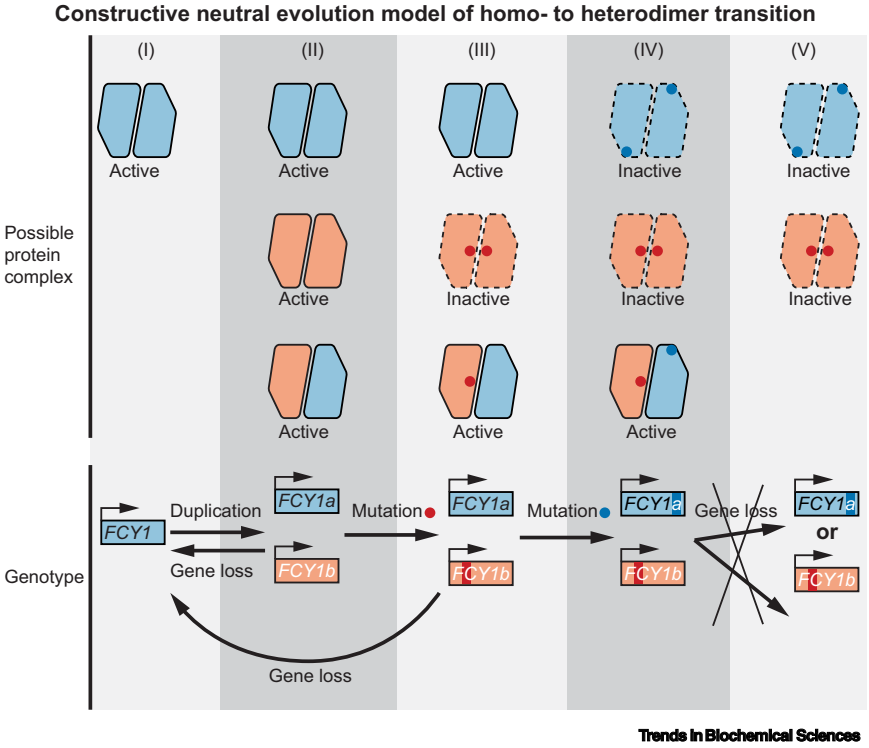

Figure 1. Constructive neutral evolution model of homodimer to heterodimer transition. At least 207 unique obligate heterodimers of cytosine deaminase (Fcy1) can potentially emerge through this process, based on the work by Després *et al.* [2]. The bottom section shows the extant *FCY1* locus in the budding yeast (I), and the loci after gene duplication or point mutations. The top section shows the possible homodimers and heterodimers that can be formed with the given loci. Després *et al.* used experimental evidence to demonstrate that the evolution from a homodimer (I) to a heterodimer (IV) can be a neutral evolutionary process because all genotypes can produce at least one form of active dimers. However, the active heterodimer at (IV) is a point of no return because individuals that lose either copy of the gene (V) can only produce inactive homodimer. No heterodimer can be formed at (V) because one gene is lost.

By demonstrating that the conditions for homodimer-to-heterodimer transition by CNE are achieved fairly readily following gene duplication, the experimental results from Després *et al.* indicate that adaptive explanations are not necessary to explain the prevalence of this transition in evolution. There are possible adaptive explanations for why a heterodimer might be superior. Yet, given Després *et al.*'s findings, the extent to which adaptation has a role in dimer complexification must be evaluated on a case-by-case basis. Importantly, however, even if the initial transition to heterodimer is nonadaptive, the increase in complexity can provide the raw material for future innovation [5,6]. With both copies under reduced constraint due to compensation from its partner, each is free to further specialize (subfunctionalization) or develop new roles (neofunctionalization) [8]. For example, a catalytically dead subunit of a dimeric kinase could form new interactions with other proteins while the active subunit specializes in catalysis [9,10]. An important open question is whether nonadaptive increases in complexity driven by CNE, followed by adaptation enabled by reduced constraint, is a general mechanism for evolutionary innovation.

It is also of interest, given the apparent leniency of the requirements for homo-to-heterodimer transition, and its irreversibility, to understand what factors might limit this process. Are all homodimers destined to become heterodimers over sufficient evolutionary time or are there costs or barriers to heterodimerization that are challenging to capture in laboratory experiments, perhaps due to variation in environment or genetic background? It is notable that, as the authors point out, *FCY1* has itself duplicated several times but has not yet become an obligate heterodimer in any known yeast lineage [2]. The pioneering experimental approach developed by Després *et al.* provides the foundation for further empirical research to probe the mechanisms and dynamics of molecular evolution.

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Declaration of interests

A-R.C. is a member of the Scientific Advisory Board for ProFound Therapeutics. The remaining authors have no interests to declare.

[1]Department of Computational and Systems Biology, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15213, USA
[2]Pittsburgh Center for Evolutionary Biology and Medicine, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15213, USA
[3]Co-first authors: these authors contributed equally to this work

*Correspondence:
anc201@pitt.edu (A.-R. Carvunis).
@X: @carvunis

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References

1. Galtier, N. (2024) Half a century of controversy: the neutralist/selectionist debate in molecular evolution. *Genome Biol. Evol.* 16, evae003
2. Després, P.C. *et al.* (2024) Compensatory mutations potentiate constructive neutral evolution by gene duplication. *Science* 385, 770–775
3. Marsh, J.A. and Teichmann, S.A. (2015) Structure, dynamics, assembly, and evolution of protein complexes. *Annu. Rev. Biochem.* 84, 551–575
4. Stoltzfus, A. (1999) On the possibility of constructive neutral evolution. *J. Mol. Evol.* 49, 169–181

5. Hochberg, G.K.A. *et al.* (2020) A hydrophobic ratchet entrenches molecular complexes. *Nature* 588, 503–508
6. Pillai, A.S. *et al.* (2020) Origin of complexity in haemoglobin evolution. *Nature* 581, 480–485
7. Finnigan, G.C. *et al.* (2012) Evolution of increased complexity in a molecular machine. *Nature* 481, 360–364
8. Innan, H. and Kondrashov, F. (2010) The evolution of gene duplications: classifying and distinguishing between models. *Nat. Rev. Genet.* 11, 97–108
9. Mace, P.D. and Murphy, J.M. (2021) There's more to death than life: noncatalytic functions in kinase and pseudokinase signaling. *J. Biol. Chem.* 296,
10. Mallik, S. *et al.* (2022) How gene duplication diversifies the landscape of protein oligomeric state and function. *Curr. Opin. Genet. Dev.* 76, 101966