

## NEWS AND VIEWS

# Genetic interactions in yeast: is robustness going bust?

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One of the main and naturally appealing tasks of systems biology is system's dissection and identification: knowing who does what to whom and when. A classical way to go about it in cell biology has been to knock out (delete) the genes of the system studied one by one and trace the effect that each such knockout has on the phenotype (the system's manifested behavior). In yeast (and in several other organisms), this procedure has led to the striking discovery that most of the genes are dispensable as they have negligible effects on the organism's growth rate and, hence, are apparently non-essential. Several mechanisms have been proposed to be responsible for this observed robustness, including the existence of duplicate genes providing backups to each other, alternative metabolic and signaling pathways and an intrinsic flexibility that stems from the need to accommodate a variety of potential growth environments (Papp *et al.*, 2004). The question of whether cellular robustness has been directly selected for as a way to avoid deleterious mutations (genetic robustness) or has evolved as a side effect of other adaptive processes such as the need to grow in different conditions (environmental robustness) has also received considerable attention (de Visser *et al.*, 2003).

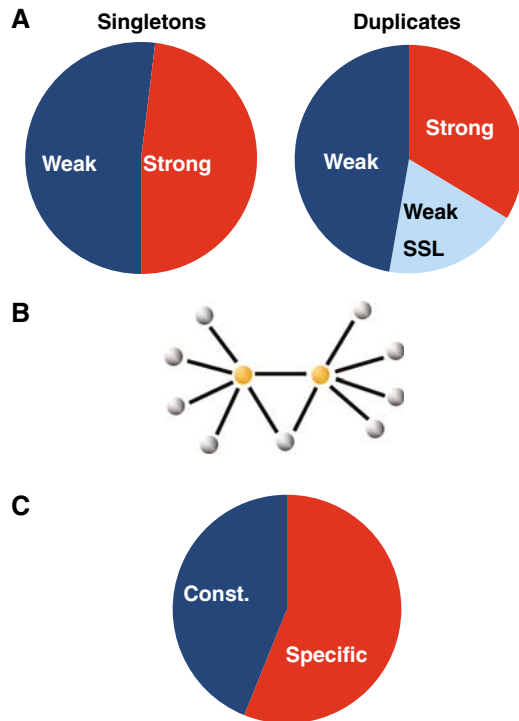
In a recent paper published in *Molecular Systems Biology*, Jan Ihmels, Jonathan Weissman and colleagues provide an in-depth study of one important facet of cellular robustness, focusing on the potential role of gene duplicates (paralogs) in providing backup compensation to each other (Ihmels *et al.*, 2007). Previous work has shown that some duplicates (but not all) do provide functional backups (Kafri *et al.*, 2005) but from an evolutionary standpoint complete backup between genes would be unstable in the long run (Brookfield, 1992). The current study utilizes a recently developed experimental approach based on the generation of high-density genetic interaction maps, termed epistatic mini-array profiles (E-MAPs), for a large set of genes (Schuldiner *et al.*, 2005). By systematically generating double knockouts of non-essential genes and assessing their fitness (measured as growth rate), it is possible to identify both positive (alleviating) and negative (aggravating) genetic interactions. Among the latter, a large range of interactions was observed, ranging from synthetic sickness (reduced growth rate) to synthetic lethal (SL) (no growth). To study the contribution of duplicates to robustness, the authors compared pairwise interactions between 92 genes that were non-essential in rich growth media

and have exactly one duplicate copy (paralog), and 300 non-essential genes lacking paralogs (singletons).

The new E-MAP data permits the study of functional interactions between duplicate pairs in a more direct and accurate manner than was previously possible. The proportion of paralog pairs with a synthetic sick/lethal (SSL) interaction between them is found to be significantly high compared to random gene pairs, providing direct evidence for duplicate buffering. However, the overall contribution of duplicate buffering to gene dispensability is rather small as the majority of gene duplicate pairs do not have an SSL interaction between them. Actually, only 25% do have such an interaction (Figure 1A). As this fraction corresponds well to the excess fitness of duplicate genes over singleton ones in an array of phenotypic studies, this implies that duplicate gene pairs without an SSL interaction between them are indeed likely not to buffer each other (i.e., they are not involved in larger sets of backup genes).

Being able to identify backup duplicates directly enabled the authors to study their features versus non-backup duplicates in a comparative manner. Quite surprisingly, it turns out that backup duplicates have a large number of SSL interactions with other genes, showing that their mutual backup is fairly limited. Notably, the interaction patterns of a pair of mutually buffering duplicate genes are markedly different from each other, indicating that they have also limited shared functionality (Figure 1B). Similarly, when sensitivity to a variety of environmental challenges is used as functional signature (Brown *et al.*, 2006), the respective sensitivity profiles of buffering duplicates appear largely uncorrelated. Interestingly, these findings parallel the previously discovered divergence of protein-protein interaction patterns of duplicate genes (Berg *et al.*, 2004). Taken together, these findings make the important suggestion that functional divergence and innovation, and not functional backup, have been the prevailing force behind the retention of gene duplicates.

Interestingly, a recent paper published in parallel to that of Ihmels *et al.* (2007) has probed closely related questions using a different methodology (Harrison *et al.*, 2007). This study has set to investigate the extent and manner by which the functional impact of single and double gene knockouts in yeast change across different growth environments. To this end, as the direct experimental testing of such an endeavor is still unfeasible, the authors employ a flux-balance analysis



**Figure 1** (A) The fraction of weak versus strong growth defects after deletion of singleton genes versus this fraction after the deletion (by single knockouts) of genes with duplicates. As evident, the deletion of the latter genes leads to weaker growth defects than the deletion of the former, testifying to their contribution to cellular robustness. (B) An illustration of the different patterns of gene interactions that typically characterize pairs of duplicate genes. (C) The fraction of genes with media-specific versus constitutive patterns of predicted SL interactions.

computational model, where they exhaustively perform all single and double knockouts of non-essential genes *in silico*, and trace their predicted outcome (in terms of growth rate and viability) across 53 different growth conditions. Focusing on SL interactions where the double knockout shows a complete no-growth phenotype, they find 98 gene pairs with a predicted SL interaction. The distribution of environmental specificity of these predicted SL interactions is markedly bimodal, with many gene pairs displaying SL interactions across most growth conditions and many on just very few of them (Figure 1C). The predictions of the model were then validated by an *in vivo* double gene knockout experiment and by a literature search. The strong context dependency of the pattern of SL interactions observed provides evidence for a correlation between environmental and genetic robustness. Furthermore, it led the authors to conclude that mutational (genetic) robustness is unlikely to be the trait directly selected for. Rather, they propose that adaptation to new nutritional conditions may drive the evolution of novel metabolic pathways and that the enhanced resistance to harmful mutations may just be a side effect of such an evolutionary drive. Interestingly, this conclusion stands in contrast to mutational robustness in microRNAs, where direct evolution of genetic robustness has been recently demonstrated (Borenstein and Ruppín, 2006).

Investigating the potential functional significance of the SL interactions discovered, Harrison *et al* find that SL interacting genes are not gained or lost together during evolution (in contrast, e.g., to genes encoding members of a protein complex), indicating that they play distinct, context-specific functional roles. This finding is akin to the finding of Ihmels *et al* that backup duplicate genes exhibit markedly different interaction patterns with other proteins. Taken together, these results show that backup relations are not simple all-or-none phenomena, but intricately vary in a complex, context-sensitive manner. This also bodes well with earlier findings showing that duplicate paralogs provide maximal backup when they have diverging expression patterns across different conditions and are only partially co-regulated (Kafri *et al*, 2005). All in all, it seems that the emerging picture is one of a delicate balance between pairs of genes diverging to assume a multitude of different functions, yet maintaining at least some level of functional backup. One promising way to gain a better understanding into the nature of genetic interactions and robustness, which we have just begun to explore, is to look deeper ‘into the heart of darkness’ by analyzing strains carrying larger sets of gene knockouts (three, four, etc.) (Deutscher *et al*, 2006).

## References

- Berg J, Lässig M, Wagner A (2004) Structure and evolution of protein interaction networks: a statistical model for link dynamics and gene duplications. *BMC Evol Biol* 4: 51
- Borenstein E, Ruppín E (2006) Direct evolution of genetic robustness in microRNA. *Proc Natl Acad Sci USA* 103: 6593–6598
- Brookfield J (1992) Can genes be truly redundant? *Curr Biol* 2: 553–554
- Brown JA, Sherlock G, Myers CL, Burrows NM, Deng C, Wu HI, McCann KE, Troyanskaya OG, Brown JM (2006) Global analysis of gene function in yeast by quantitative phenotypic profiling. *Mol Syst Biol* 2:1
- de Visser JGM, Hermisson J, Wagner GP, Meyers LA, Bagheri-Chaichian H, Blanchard JL, Chao L, Cheverud JM, Elena SF, Fontana W, Gibson G, Hansen TF, Krakauer D, Lewontin RC, Ofria C, Rice SH, von Dassow G, Wagner A, Whitlock MC (2003) Perspective: evolution and detection of genetic robustness. *Evolution* 57: 1959–1972
- Deutscher D, Meilijson I, Kupiec M, Ruppín E (2006) Multiple knockouts analysis of genetic robustness in the yeast metabolic network. *Nat Genet* 38: 993–998
- Harrison R, Papp B, Pal C, Oliver SG, Delneri D (2007) Plasticity of genetic interactions in metabolic networks of yeast. *Proc Natl Acad Sci USA* 104: 2307–2312
- Ihmels J, Collins SR, Schuldiner M, Krogan NJ, Weissman JS (2007) Genetic interactions reveal the true cost of gene loss for singleton and duplicate genes. *Mol Syst Biol* 3:86
- Kafri R, Bar-Even A, Pilpel Y (2005) Transcription control reprogramming in genetic backup circuits. *Nat Genet* 37: 295–299
- Papp B, Pál C, Hurst LD (2004) Metabolic network analysis of the causes and evolution of enzyme dispensability in yeast. *Nature* 429: 661–664
- Schuldiner M, Collins SR, Thompson NJ, Denic V, Bhamidipati A, Punna T, Ihmels J, Andrews B, Boone C, Greenblatt JF, Weissman JS, Krogan NJ (2005) Exploration of the function and organization of the yeast early secretory pathway through an epistatic miniarray profile. *Cell* 123: 507–519