Robustness Mechanisms in Biology

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1 Introduction

Robustness is an important concept within the biological world. We might define it by the *small variance of a state* of the subject, e.g. health, fitness or phenotype, *against changes in the underlying working conditions* compared to the variance of other possible states against the same changes.
Robustness differs from other concepts like stability and adaptivity (returning to a desired state), persistence, recovery and flexibility by its intrinsic structural components. Robustness may include topics like stability (small state perturbations lead to only small state changes) or structural stability (small structural parameter changes lead to a new system with the same qualitative behaviour), but include more aspects like organisation and architecture of a system, the offset between function, possible functional changes and architecture, and topics like the controversy between adaptivity and identity, i.e. plasticity vs. stability.

In our context we are mostly interested in mechanisms of robustness in the molecular biological world. We are interested in *phenotypic robustness*, i.e. the robustness of biological entities against changes in the underlying environmental and genetic mechanisms. There are two main robustness principles known in literature: canalization and neutrality.

- *Canalization*: During the developments of a species, phenotype variations might be suppressed. This was first formulated by Waddington [1] and can be seen as a multi-hill potential fitness landscape where a ball rolls always downhill. Although there are several possible routes, only one (the channel) with the best fitness is taken during species development. Changing environmental conditions may lead to changes in the landscape, altering the resulting phenotype. Nevertheless, the phenotypic variance in many developments will stay small.

- *Neutrality*: Although there might be huge phenotypic differences, the fitness of those phenotypes may not differ significantly and will not lead to natural selection; they are evolutionary neutral. This idea was introduced by Kimura [2][3] and first observed for electrophoretic data for a species which had a much bigger variance than expected [4].

The molecular reasons for these observations are manifold. Mechanisms are based on

- *Redundancy*: The effects of multiple copies of a gene (*paralogues* copies during replication) are buffered because doubling the function promote may not lead to higher resulting effect concentrations. This is true for transcription factors, signal transduction proteins, metabolic pathway genes and the variable genes encoding antibody peptides. Thus, the effects of mutations of the paralogues genes are buffered, leaving the original phenotype intact and providing phenotypic robustness. This mechanisms is only counteracted by its implied molecular costs which are too high for fast replicating and translating organisms like viruses and bacteria.

- *Deleterious variance suppression (anti-redundancy)*: Even if the molecular costs are not so important, high redundancy may lead to accumulation of even mild deleterious mutations after several generations. This was called "Muller's ratchet" [5]. Therefore, counter-mechanisms to redundancy have to be implemented.

There are several mechanisms known for suppressing the deleterious mutations. They can be grouped into two categories: those who deal with the mutations at the basic kernel level and repair the aberrations, and those who try to fix the results of the mutations.

(a) *Robustness by mutation repair and buffering:* Since the DNA copy mechanisms are not very accurate and subject to many irritations, already the central cell building and replication mechanisms contain some repair mechanisms. Beside the removal and replacement of damaged regions

(*excision repair*), the replacement of noncomplementary bases of opposite strands in the double helix (*mismatch repair*) and the reversal of nucleotide damage (*direct repair*), there is a special mediator for the messenger RNA (mRNA) code units (*codon*) sequence recognition (tRNA), which is able to detect nonsense codons and will then suppress the tRNA [6]. Also, all messenger RNA which transfers the transcripted DNA information to the protein synthesis place in the cell, is supervised [7] and subject to degradation by the *nonsense-mediated* mRNA *decay* (NMD) process.

Another mechanism exists in kernel based cells (*eukaryotes*) and multi-cellular subjects. There, so called checkpoint genes (e.g. p53) stop the development of the cell until all damages are repaired and then let the development continue. For instance, p53 is concentrated in cells with erroneous cell products. There, it stops the cell evolution and triggers the DNA repair mechanisms. After this, the concentration fades away and let the cell proliferate. If the damage is too high, p53 will cause the cell death (*apoptosis*) instead. This is also cause by other *tumor necrose factors* (TNF).

Masking of gene mutations (buffering) is achieved by comparing the DNA strings and methylation of differences (*methylation induced promeiothically* MIP). This process of silencing defective genes is called *imprinting* [11].

(b) Robustness by mutation result repair and buffering: Mutations may be observed indirectly by misfolded or mis assembled proteins. Here, a protein quality control by special proteins (*chaperons*) located in the endoplasmic reticulum (ER) checks all passing proteins and induces an ER-associated protein degradation. This prevents the accumulation of unfolded proteins [8].

Another mechanism for clearing the mutational results is *autophagy* - the breaking and recycling of translated protein products of cancer-inducing genes (*oncogenes*) putting them into double-membrane cell objects (vesicles) called *autophagosomes*, breaking the content down by hydrolases. This is caused by overexpression of the gene *beclin* [9].

There is also the possibility of dominating the deleterious results by internal regulation. Dominant genes may act by so called *dominance modifiers* on the kinetic parameters of enzymatic pathways, correcting the influence of defective genes [10]. This seems to be one of the main reasons for organisms with two chromosome sets (diploid) that single mutations (alleles) do not lead to phenotypic effects.

(c) Robustness by mutation result enhancement: A complete opposite strategy to robustness increase by repair is the strategy of *enhancing* all damages of mutations. This leads to enhanced fitness variance and therefore to natural selection. There are different mechanisms to do this.

One of them is the programmed death of cell lines in long living individuals. Ever-living cells will accumulate all mutations. Therefore, the cleaning of cell ensemble of mutated ones is an important feature. For this, the chromosomes contain caps on their ends, the *telomeres*. On each reproduction of the mature cell, the telomeres loss a part of them. If too much is lost, the splitting of the mitotic cell will be arrested. This puts an end to the lifetime of all somatic cells.

Another one is the loss of key error repair genes in mitochondria which leads to a reduced rate of accumulation of deleterious mutations.

All robustness mechanisms can be grouped by other criteria, too. For instance, we might distinguish between mechanisms which act directly on the genes like the DNA or RNA repairing mechanisms, and mechanisms which are only statistically valid for cell lines or generations of populations. There are robustness mechanisms which act purely on the statistics of population genetics, for instance the *codon bias*. Here we have the fact that each code unit in the DNA, the *codon*, is built up by a triplet of the four nucleotides A,G,C,T. This gives us $4^3 = 64$ possible codons. Each codon encodes one of the 20 amino acids. Thus, we have more possible codons than amino acids to code, approximately 3 per acid. We might expect an equal occurrence of each the four nucleotides, but this is not true. Each species has its preferences for certain codons giving a bias. Therefore, uniform distributed mutations can be distinguished statistically from the species and be exposed to internal or external phenotype selection.

Another statistical robustness mechanism is the so called *genetic bottleneck*. All generation transformation which reduces (*haploid*) and enhances (*diploid*) the number of chromosomes (genes) will in the reduced phase expose the previous masked mutations to selection. Small populations with small selection and small offspring have to have a large bottleneck in order to strip off the deleterious muta-

tions, whereas large populations with many offspring and high natural selection need only a small bottleneck.

2 Metabolic pathway robustness

A famous example for molecular canalization is the plasmid ColE1 replication of the *Escherichia coli* bacterium. Here,

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