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Genomic Stress Responses Drive Lymphocyte Evolvability: An Ancient and Ubiquitous Mechanism

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Somatic diversification of antigen receptor genes depends on the activity of enzymes whose homologs participate in a mutagenic DNA repair in unicellular species. Indeed, by engaging error-prone polymerases, gap filling molecules and altered mismatch repair pathways, lymphocytes utilize conserved components of genomic stress response systems, which can already be found in bacteria and archaea. These ancient systems of mutagenesis and repair act to increase phenotypic diversity of microbial cell populations and operate to enhance their ability to produce fit variants during stress. Coopted by lymphocytes, the ancient mutagenic processing systems retained their diversification functions instilling the adaptive immune cells with enhanced evolvability and defensive capacity to resist infection and damage. As reviewed here, the ubiquity and conserved character of specialized variation-generating mechanisms from bacteria to lymphocytes highlight the importance of these mechanisms for evolution of life in general.

1. Introduction

Due to their commitment to somatic duties, bodily cells have a limited ability to evolve during the lifetime of the individual.[1,2] Instead of pursuing their own replicative agendas these cells are "de-Darwinized" to serve reproductive interests of the organism as a whole.[3,4] However, to match pathogens in their potential to rapidly alter their heritable characteristics, a class of somatically evolving immune cells appeared in early vertebrates to undergo genetic diversification and selection and to adapt to the antigenic environment of the host.[5–7] Clones of these adaptive immune cells, known as lymphocytes, exist in vertebrate organisms and expand, differentially, depending on the antigen binding fitness of their receptors.[8,9] Thanks to their adaptive evolution, developing immune cell clones mediate a variety of functions from protection against pathogens to maintenance of self-structures.[10]

Despite our increased understanding of somatic evolution of lymphocytes, little is known about how these cells acquired their potential to evolve, especially that the notion of evolution of evolvability is still under dispute.[11] Understood as an ability to produce heritable adaptive variation, evolvability of lymphocytes depends on specialized variation generating mechanisms, such as V(D)J recombination, immunoglobulin gene conversion (IGC), class switch recombination (CSR), and somatic hypermutation (SHM). Here we suggest that these variation-generating mechanisms have been co-opted en masse by lymphocytes from ancient stress-response DNA modification systems allowing these cells to gain potential to evolve somatically during the lifetime of the host. The ancient mutagenic and recombination mechanisms are increasingly recognized as main drivers of adaptive changes in cell populations from bacteria to cancer and considered targets for specialized anti-evolvability therapy to arrest evolution of these cells.

2. Molecules Involved in Antigen Receptor Gene Diversification Are Highly Conserved

Antigen receptor gene diversification relies on the activity of ubiquitous recombination and DNA repair enzymes (**Table 1**).[12] As many of these enzymes mediate essential cellular processes, helping to maintain integrity of DNA, they are conserved from bacteria to humans.[13]

One class of conserved enzymes that mediate antigen receptor gene diversification are error-prone polymerases.[14,15] This includes Polη that sharing homology with RAD30 in *S. cerevisiae* and with dinB in *E. coli* plays a major role in generating mutations during SHM and IGC.[16] Other polymerase, related to yeast REV3 and bacterial polymerase B family members, Polζ is involved in SHM and CSR.[17,18] Terminal deoxynucleotidyl transferase (TdT), which mediates random addition of N-nucleotides at the junction between rearranged V, D, and J gene segment provides still another example of a homology between error-prone polymerases in lymphocytes and in unicellular organisms. This enzyme is a member of X-family of polymerases and its ortholog can be found in early deuterostomes.[19,20] Hence, error-prone polymerases that mediate SHM, IGC, and V(D)J recombination are closely related to similar enzymes in bacteria, archaea, and simple eukaryotes.

DNA repair enzymes involved in antigen receptor gene diversification are also conserved.[21] This includes members of Ku family that play an important role in non-homologous end joining (NHEJ) repair during CSR and V(D)J recombination and whose ancient homologs can be found in bacteria and yeast.[22]

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Table 1. Examples of bacterial and yeast homologs of genes involved in antigen receptor gene diversification.

The enzyme type	Bacteria	Yeast	Higher vertebrates	The role in antigen receptor gene diversification
Polymerases	A-Pol family	–	Polθ	IGC in chicken; SHM
		–	Polν	IGC in chicken
	B-Pol family	Polζ	Polζ	SHM
	X-Pol family	Pol IV	TdT	N-nucleotides addition during V(D)J recombination
			Polμ	NHEJ during V(D)J recombination addition of P-nucleotides)
		Pol IV	Pol λ	NHEJ during V(D)J recombination (addition of P-nucleotides)
	Y-Pol family	RAD30		
	Polη (RAD30A)	IGC in chicken; SHM		
			Polι (RAD30B)	SHM
		REV1	REV1	IGC in chicken, SHM
Mismatch repair enzymes	MutS	MSH2	MSH2	Mismatch repair during SHM, IGC in chicken
	MutS	MSH6	MSH6	
NHEJ proteins	Ku	YKu70	Ku70	NHEJ during V(D)J recombination
	Ku	YKu80	Ku80	
	–	–	DNA PK	
	–	LIF1	Xrcc4	
	–	Dnl4	DNA ligase IV	
	–			
	Nej1	Cernunnos (XLF)		
HR repair factors	RecA	Rad51	Rad51	Orderly assembly of homologous DNA strands during IGC
	RecO	Rad52	Rad 52	
Nucleases	UDG	UDG	UDG	Excision of uracil following AID action (SHM, CSR, IGC)
	–	Pso2p	Artemis	Hairpin opening during V(D)J recombination
	–	EXO1	EXO1	MMR-mediated strand degradation during SHM, switch region joints formation during CSR
	Exonuclease III	APN2	APE1	Producing SSB during CSR. Producing a single strand break during IGC
Sensors of DNA damage	UvrA UvrD	Rad3 Tel1 MEC1	ATM	Sensing DNA damage during V(D)J recombination and CSR
Deaminases	–	–	AID	Cytosine deamination during CSR, SHM, IGC
Domesticated transposases	–	–	RAG1/2	Production of DNA breaks during V(D)J recombination

Components of another repair cascade, homologous recombinational (HR) repair pathway, which mediate IGC are also conserved as illustrated by the phylogenetic relationship between Rad51/Rad52 in mammals and Rad51/Rad52 in yeast.[23] Finally, components of the mismatch repair (MMR) pathway like MSH2/MSH6, whose aberrant activation helps to generate mutations at A-T pairs downstream of activation induced deaminase (AID)-mediated lesion appear to be related to yeast MSH2/MSH6 as well as to bacterial MutS.[24]

Endonucleases and exonucleases cleaving DNA during SHM, CSR, and IGC make up still another group of conserved, gene-altering molecules. For example, uracil-DNA glycosylase (UNG), which helps to excise uracil following the activity of AID, can be already found in bacteria and yeast.[25] APE1, which produces single stranded breaks following the activity of UNG during CSR and IGC exhibits high level of sequence identity to yeast APN2 and bacterial exonuclease III.[26,27] Finally, despite appearing only recently in phylogeny, Artemis endonuclease, which helps to open a hairpin structure during V(D)J recombination, is related to Pso2p that participates in NHEJ in yeast.[28]

In contrast to the above, AID and RAG1/2 appeared only in multicellular organisms. However, even these molecules are distantly related to proteins of unicellular species as evidenced by the origin of AID from bacterial t-RNA editing enzymes and by the likely transfer of these enzymes from bacterial toxin systems into metazoan organisms.[29] Molecules closely related to AID have been identified in sea urchins and in brachiopods and sequence analyses point to existence of related deaminases also in slime molds and algae.[30–32] RAG proteins, whose crystal structure has been recently determined, are closely related to viral or

bacterial *Transib* transposases.[33–35] Already identified in protostomes and invertebrate deuterostomes like the purple sea urchin and amphioxus, homologs of RAG1/RAG2 proteins well precede the appearance of adaptive immunity in vertebrates.[31,36–38] Hence, components of variation-generating mechanisms in lymphocytes are closely related to enzymes involved in DNA processing in unicellular species and many of these molecules can be found in multicellular organisms that do not have lymphocyte-based immunity.

3. Hypermutation and Programmed Gene Recombination Mediate Genomic Stress Responses from Bacteria to Humans

Variation generating processes such as hypermutation, gene conversion, and transposase-based gene rearrangement are not unique to lymphocytes but are utilized by many types of cells to enhance their adaptation during stress.[39] Despite the exact relationships between these variation generating mechanisms across the whole tree of life are not well understood, common themes in the evolution of these mechanisms can be identified as many of them depend on DNA damage, error-prone repair, and transposon domestication to mediate randomization of genetic material and to enhance adaptation of the corresponding cell population to altered conditions. Indeed, by increasing genetic diversity during stress, these mechanisms act as evolvability enhancing systems accelerating emergence of novel adaptive solutions in the populations during adverse environmental conditions.

3.1. Stress-Induced Mutagenesis in Bacteria

In bacteria, a prominent example of stress-induced evolvability enhancing system is hypermutation.[40] In *E. Coli* this form of mutagenesis depends on SOS DNA damage response and a general (RpoS) stress response to upregulate and license the activity of error-prone polymerases of the Y family (Pol IV and Pol V) that process DNA during HR repair.[41] DNA errors resulting from the activity of these enzymes escape MMR, as the function of the MMR pathway is altered during stress due to downregulation of MutS and MutH proteins.[42,43] Another strategy, which is used by some bacteria to elevate mutagenesis during stress involves a switch from HR to NHEJ pathway, which promotes error-prone DNA repair due to its associated nucleotide trimming and filling in.[44] In addition to the hypermutation programs, regulated recombination mechanisms help to increase genetic variation in some bacteria to enhance adaptability of these cells to adverse environmental conditions. This includes gene conversion in *Neisseria gonorrhoeae* and *Borrelia burgdorferi* that relying on a process of nonreciprocal exchange of genetic information can evade immune recognition by the hosts.[45–47] Thus, bacteria rely on HR and NHEJ to increase the rate of genetic changes and to improve their chances of survival in adverse environmental conditions.

3.2. Stress-Induced Mutagenesis in Unicellular Eukaryotes

Unicellular eukaryotes also rely on adaptive mutagenesis to enhance their adaptation to stress.[48] This includes hypermutation, which in the case of *Saccharomyces cerevisiae* depends on error-prone polymerases and NHEJ pathway to introduce changes in the DNA.[49] Engaging these molecules and this repair pathway, the adaptive mutagenesis systems of *S. cerevisiae* are deeply conserved, sharing many characteristics with similar mechanisms in prokaryotes.[50] Further highlighting evolutionary links with stress-induced mutagenetic systems in bacteria is the observation that the MMR pathway in *S. cerevisiae* is altered during stress to promote, rather than to correct nucleotide mismatches during altered environmental conditions.[51] Furthermore, stress-induced mutagenesis in yeast, again, like in bacteria, is not limited to point mutations as gene recombination programs are also activated in these organisms during altered conditions. This includes recombination mediating mating type switching in *Kluyveromyces lactis* and stress-induced enhancement of somatic recombination in the macronucleus of *Tetrahymena thermophila*.[52,53] In addition to these types of gene recombination, both mediated by domesticated transposases, some protozoan parasites like the abovementioned bacterial pathogens, activate gene conversion mechanisms to enhance their adaptation to immune host environment.[54] While the impact of host-derived stressors on the frequency of antigen switching in parasites like *Trypanosoma brucei* still needs to be determined, evidence exists that living conditions greatly impact on the rate of antigenic variation in this parasite.[55] In short, unicellular eukaryotes like bacteria utilize hypermutation and programmed gene rearrangement to increase their variation and to enhance their adaption to unfavorable environmental conditions.

3.3. Stress-Induced Mutagenesis in Multicellular Eukaryotes

Stress-induced variation generating mechanisms are not restricted to unicellular organisms as multicellular eukaryotes also activate their mutagenic programs to increase variation of their progeny during stress. This is evident in organisms that propagate asexually like *Daphnia* in which metal-related stressors (copper and nickel) increase the rate of inheritable genetic changes in the offspring.[56] An increase of mutagenic rate is also apparent in *C. elegans,* which exposed to heat upregulates its germline diversity.[57] In *Drosophila,* nutrition-related stress interferes with the fly oocyte's capacity to repair DNA breaks in the sperm following fertilization; an alteration that leads to increased genomic diversity and adaptability of the progeny.[58] Hence, in multicellular organisms, like in microbes, the rate of mutagenesis is greatly increased during stress promoting genetic variation and adaptability of the organisms' descendants.

More strikingly, stress-induce diversification systems also operate post-zygotically, in somatic cells, even in organisms with firm germline/soma separation.[59] This is evident in mammals, which like stressed bacteria and yeast activate an error-prone NHEJ pathway rather than high fidelity HR pathway to process double stranded breaks (DSB) during hypoxic stress.[60–61] Further highlighting similarities with the mutagenic response systems of microbes is downregulated expression of MLH1 and PMS2 (homologs of bacterial MutL) and aberrant activation of the MMR in mammalian cells during hypoxia.[62] Adaptive mutagenesis of somatic cells has been extensively investigated in the

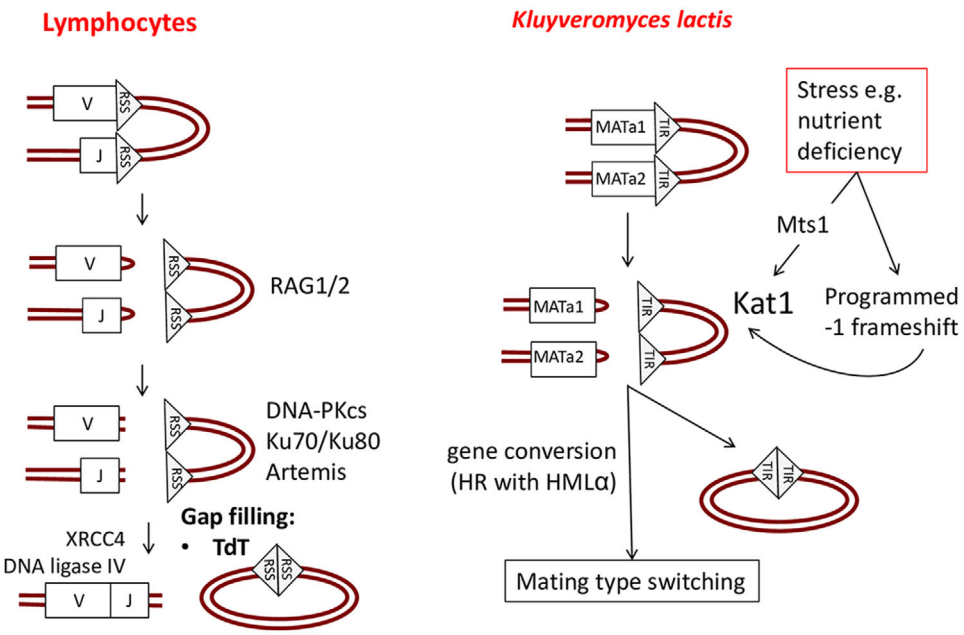

Figure 1. Transposase-mediated recombination and repair in lymphocytes and in yeast. a) In lymphocytes, V(D)J recombination is mediated by RAG1/2 that produce double stranded breaks at RSSs. The process is followed by NHEJ and looping out of the intervening DNA fragment. b) To produce mating-type switching during stress, Kluyveromyces lactis, uses a domesticated transposase Kat1, which despite unrelated to RAG1/2, also produces double stranded breaks at defined signal sequences (terminal inverted repeats TIR); a process, which also depends on hydroxyl nucleophilic attack, hairpin cup formation and looping out.

context of cancer cells, which have been found not only to block their high-fidelity HR pathways and to alter their MMR pathway but also to upregulate expression of error-prone polymerases like Polκ, Polι and Rev1 (homologs of bacterial Pol IV and Pol V).[63] All of this reveals that stress-induced mutagenic mechanisms in mammals are profoundly similar to those that are utilized by bacteria and yeast.

The presence of stress-induced mutagenic systems in somatic cells raises the question of their adaptive advantage for the organism. At the face value, upregulation of mutagenesis during stress seems potentially detrimental, elevating risk of cancer and functional loss.[64] However, it is emerging that thanks to their potential to promote somatic cell evolution, genomic variation may play important physiological functions in the organism enhancing fitness of bodily organs and tissues.[65] For example, studies of stress-induced liver adaptation indicate that karyotypic variation serves as a raw material for natural selection to permit expansion of injury-resistant clones that protect the organ from damage.[66] In the brain, especially in stem cells and progenitor cells of the hippocampus, high rate of recombination events have been observed, and many of these genetic exchanges were found to be activated by stress-associated factors to shape neuronal plasticity and to contribute to adaptive changes in cognition and behavior of the host.[67,68] The idea of somatic cell evolution has been considered already by Metchnikoff, for whom not only immune cells but all cellular linages in the organism were engaged into a Darwinian struggle during the lifetime of the organism.[69] These early views are now revivified in the context of genomic studies suggesting that increased variation is not necessarily detrimental but may also promote adaptive changes in the organism.

4. Diversification Mechanisms in Lymphocytes and in Microbes are Strikingly Similar

Stress-induced hypermutation and gene recombination programs parallel those that mediate antigen receptor gene diversification in lymphocytes. This is manifested among other things in their shared reliance on DNA lesions that act as triggers of mutagenic processing as well as in their involvement of homologous error-prone polymerases, homologous mutagenic NHEJ pathways and altered MMR pathways. Comparison between variation generating systems of microbes and lymphocytes helps to reveal analogies and homologies between these systems.

4.1. V(D)J Recombination Resembles Transposase-Based Recombination in Microbes

Striking parallels exist between the V(D)J recombination in lymphocytes and stress-induced mating type switching in *K. lactis* (**Figure 1**). Both types of rearrangement depend on domesticated transposases and both utilize clusters of gene segments to produce alterations in functional genes.[33,70] In the case of V(D)J recombination, which mediates rearrangement of antigen receptor gene segments to generate primary adaptive immune repertoire of lymphocytes in the bone marrow, the process of diversification depends on a domesticated transposase RAG1/RAG2.[71] These enzymes produce DSBs at conserved recombination signal sequences (RSSs), which are remnants of ancient terminal inverted repeats (TIRs).[33,72,73] In the case of mating type switching from MATα to MAT**a** in *K. lactis*, the process is mediated by a different transposase, Kat1, which like RAG1/RAG2 in

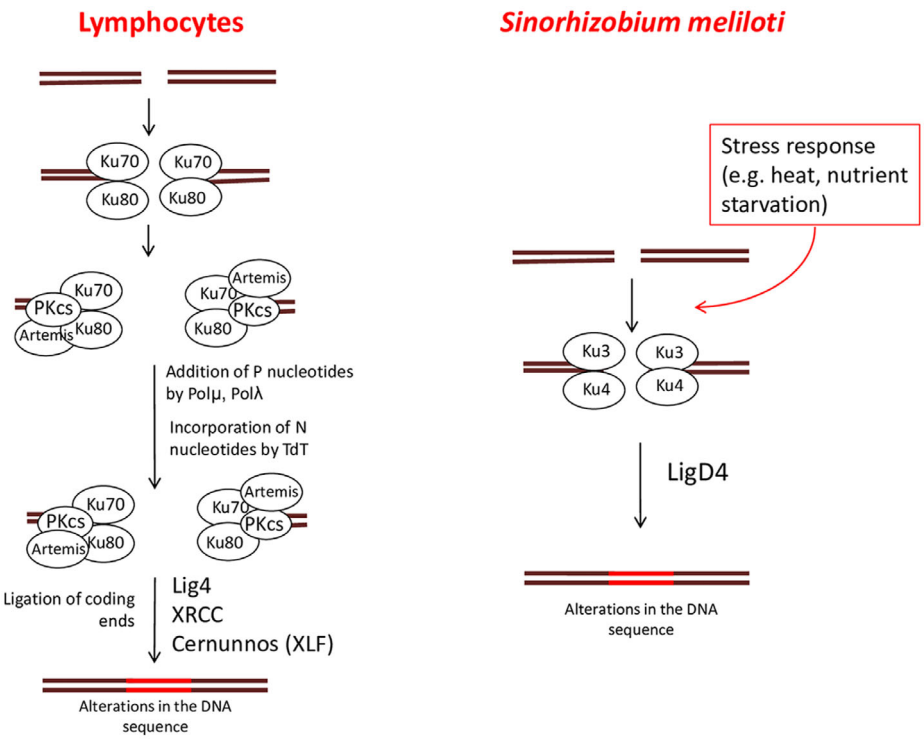

Figure 2. NHEJ in lymphocytes and in bacteria. a) Following V(D)J recombination in lymphocytes, a coding joint is formed at the junction between rearranged gene segments. Formation of this joint depends on the activity of NHEJ pathway, which involves Ku70/80 and ligase IV that seals the broken ends. b) In Sinorhizobium meliloti NHEJ is mediated by homologs of human Ku proteins, Ku3, Ku4, that, like Ku70/80 in lymphocytes, bind to broken DNA ends to initiate the end joining repair pathway. Central to this pathway is the activity of LigD4, which like ligase IV in lymphocytes helps to seal the broken DNA ends.

lymphocytes, also produces DSBs targeting conserved signal sequences in the form of TIRs.[52] In this yeast, again like in the immune cells, single-stranded nicks are produced at these motifs to allow the exposed 3′-OH groups to attack opposite DNA strands and to form characteristic hairpin cups at the ends of the breaks.[74,75] In both types of cells, the excised DNA fragments are looped out and deleted from the DNA and harpin structures are opened by dedicated endonucleases.[70] Thus, despite relying on unrelated, *Transib* and *hAT* transposases,[76,77] programmed gene rearrangements in stressed *K. lactis* and developing lymphocytes are almost identical. In short, despite being domesticated only recently, RAG1/RAG2 enzymes share many features with microbial cut-and-paste transposases and the whole process of recombination is striking similar to a stress induced mating type switching in yeast.[78]

4.2. Junctional Diversification in Lymphocytes and Microbes Is Very Similar

Junctional diversification in lymphocytes and in stress-activated microbes is very similar too (**Figure 2**). As a part of V(D)J recombination in lymphocytes, this diversification process follows recognition of DNA break by ataxia-telangiectasia mutated (ATM) kinase and depends on conserved NHEJ proteins such as Ku70/Ku80, DNA PK, XLF, Artemis, and XRCC4-DNA ligase IV.[79,80] Accompanied by error-prone polymerases (in particular, an X family member, TdT), the NHEJ in gene recombining lymphocytes is responsible for introducing variation at the coding joint of an antigen receptor gene.[81] Despite a significant phylogenetic gap between vertebrates and unicellular species, the process is almost identical to one mediating stress-induced diversification in some bacteria and yeast.[82,83] In *S. meliloti*, which is a gram-negative bacterium involved in symbiosis with leguminous plants, genomic stress induces activation of an error-prone NHEJ pathway that depends on the activity of Ku3/Ku4 proteins, which manifesting sequence similarity to the mammalian Ku70/Ku80, help to mediate genetic diversification in the bacterium.[84] NHEJ pathway also enhances genomic diversity in yeast, which relying on Yku70-Yku80 (homologs of the mammalian Ku70/Ku80 proteins), MEC1 (an ortholog of ATM/ATR) and other conserved enzymes like Dnl4, Lif1 and Lig4 helps to generate imprecise DNA junctions in these cells.[85] In terms of accuracy, stress-induced NHEJ in microbes matches that in lymphocytes in so far as it generates junctional diversity and contributes to an increased adaptation of the cell population to adverse conditions.

4.3. SHM Resembles Hypermutation in Microbes

SHM is still another antigen receptor gene diversification mechanism paralleling ancient evolvability enhancing systems (**Figure 3**).[86] In activated B cells and in certain types of shark T-cells, hypermutation generates point mutations in variable regions of rearranged V(D)J genes.[87–89] This process is mediated by AID, which—in the course of deaminating cytosine

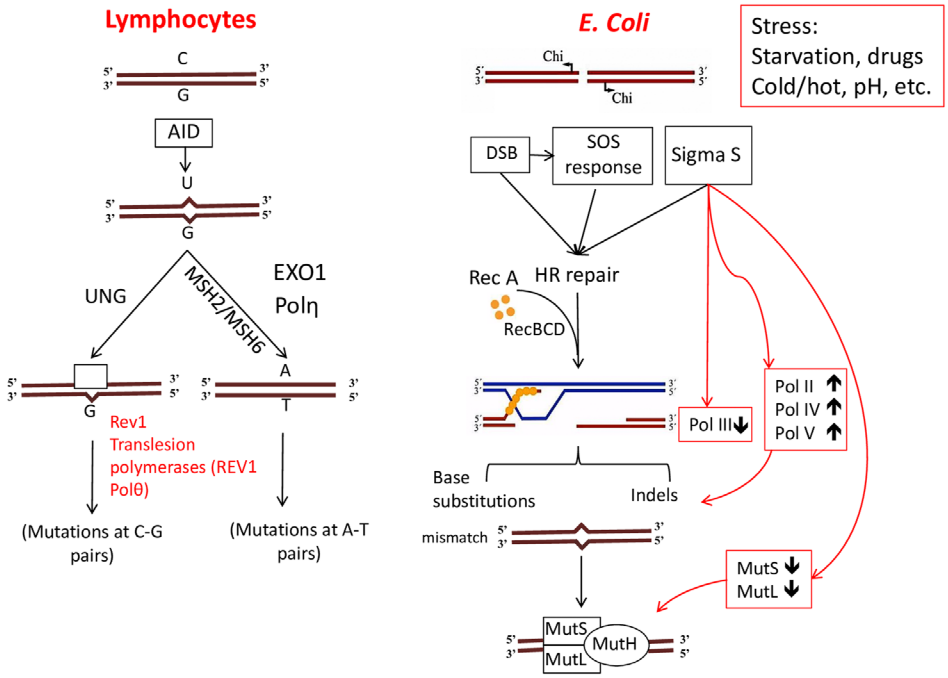

Figure 3. Hypermutation in lymphocytes and in bacteria a) In lymphocytes, SHM depends on AID-mediated lesion, which triggers activation of UNG and other DNA processing enzymes including error-prone polymerases to produce point mutations in the rearranged V(D)J genes. b) In bacteria, hypermutation is activated during stress and depends on error-prone Pol IV as well as Pol II and Pol V. Similar to SHM, mismatch repair systems in the stressed hypermutated microbes are altered to preserve DNA errors generated during the hypermutation process. In *E. Coli*, the general stress response has been found to downregulate expression of MutS and MutH (homologs of mammalian MSH2 and MSH6) to enhance variation of genes during altered environmental conditions.

at hotspots of rearranged V(D)J genes—produces lesions to be processed by mutagenic repair pathways.[90] Initiated by a DNA lesion and dependent on error-prone repair, hypermutation in microbes is strikingly similar to that in lymphocytes.[39,91] Both types of hypermutation depend on downregulation of high-fidelity polymerases (Pol III in *E. coli* and Polβ in lymphocytes) and require activation of error prone polymerases (Pol II, Pol IV, Pol V in *E. coli* and Polη, Polθ, Polι, and Polζ in lymphocytes).[17,92] Further highlighting similarities between hypermutation in bacteria and in lymphocytes is their preferential targeting of RNA:DNA hybrid structures (R-loops), which help to draw the mutagenic machinery to their genetic targets.[93,94] SHM in lymphocytes also parallels hypermutation in bacteria in that it is associated with an altered MMR system to preserve errors generated during the mutagenic process.[87] In fact, deletions of genes coding for components of the MMR pathway suppresses SHM in mice (especially at A–T pairs) suggesting that rather than repairing DNA, this cascade increases an error rate during affinity maturation of lymphocytes.[95] In short, stress-induced hypermutation in bacteria and SHM in lymphocytes share many features. Both types of mutagenesis depend on errors in DNA processing and are associated with altered MMR pathways.

4.4. IGC Resembles Gene Conversion in Microbes

IGC, which is the main antigen receptor gene diversification mechanism in birds, rabbits and farm animals is also traceable to microbial, variation generating systems (**Figure 4**). Involving nonreciprocal exchange of genetic material from pseudogenes to functional loci, the mechanics of gene conversion in lymphocytes and in bacteria is almost identical.[96,97] In the case of lymphocytes, the recombination process is initiated by an AID-mediated single-stranded nick and is followed by the activity of MRN (MRE11/RAD50/NBS1) complex as well as HR enzymes like RAD51 and BRCA2 to mediate homology-directed repair in which ψV pseudogenes act as donors of DNA fragments to be inserted into the target V region.[98] In bacteria the process of gene conversion is very similar, also initiated by a DNA lesion and followed by a homology-based transfer of genetic fragments from noncoding pseudogenes to the coding, functional region.[99] In the case of *N. gonorrhoeae* donors of DNA fragments during the conversion are pilS fragments that are copied and pasted randomly into the expressed pilE locus, which encodes type IV pili (Figure 4b). Further highlighting similarities between IGC and gene conversion in microbes is the involvement of homologous enzymes in these processes as illustrated among other things by the role of RecA homologs, which play important role in IGC (RAD51) and in gene conversion of microbes.[99,100] Similarities between antigenic variation in unicellular parasites and antigen receptor gene variation in lymphocytes are also manifested in the role of G4 heteroduplex motifs, four-stranded structures, which act as breakpoint sites for gene conversion in *N. gonorrhoeae* and *T. brucei* and which are also known to operate as targeting sites for AID during CSR.[101–104] Furthermore, allelic exclusion mechanisms are in place in lymphocytes and in *T. brucei* to ensure clonality of antigen receptors and variant surface glycoproteins

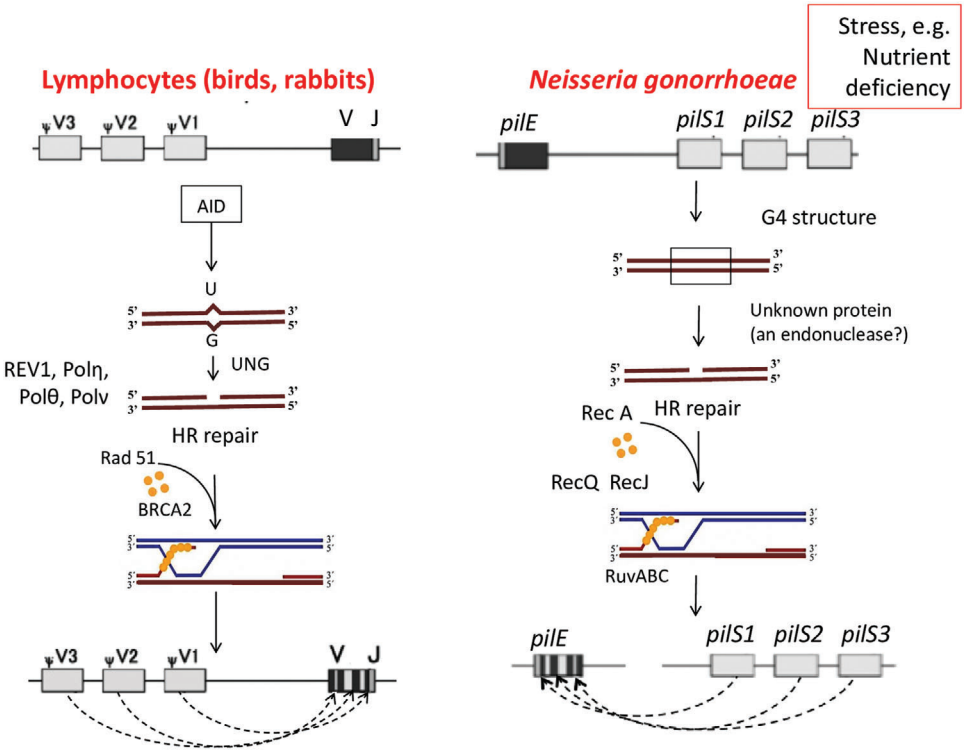

Figure 4. Gene conversion in lymphocytes and in bacteria. a) In lymphocytes of birds, rabbits and farm animals IGC depends on the activity of AID and involves homologous recombinational repair to use multiple ψV gene segments as donors of genetic information and to insert this information into antigen genes. b) Neisseria gonorrhoeae utilizes multiple pilS pseudogenes as templates to diversify genes coding for functional type IV pili. PilS pseudogenes act as templates of short DNA fragments to be integrated randomly into an expressed pilE. N. gonorrhoeae utilize conserved HR enzymes like RecA (a homolog of RAD51) to complete the process of recombination.

(VSG), accordingly.[105–107] Thus, relying on conserved molecules and homologous repair pathways, gene conversion in lymphocytes and gene conversion in unicellular parasites are fundamentally equivalent.

While similarities exist between antigen receptor gene diversification mechanisms in lymphocytes and genomic stress response in bacteria and other organisms, there are also differences between these systems. Most notably, stress-induced mutagenesis operates on a large genomic scale affecting many genes and other DNA regions, whereas SHM and programmed gene recombination in lymphocytes are focused on defined gene loci, operating in specialized tissues during defined stages of development of these cells.[108] However, stress-induced variation is not completely random either as many forms of this diversification are activated during altered conditions, focusing their action on regions surrounding DNA breaks and preferentially targeting R-loops during hypermutation.[109,110] Furthermore, as highlighted above, antigenic variation, mating type switching, and other recombinational processes in unicellular organisms are also quite specific, targeting defined gene regions and adjusting their activity to environmental conditions.[97,111] Hence, despite acting on a larger scale and lacking precise regulation characteristic of antigen variation systems, stress-induced mutagenesis is also regulated to an extent to target certain gene regions and to respond to defined changes in the environment.

5. Adaptive Immune System Co-Opts Gene Diversification Mechanisms as Evolvability Enhancers

As one can see, hypermutation, gene conversion, junctional diversification, and transposon-mediated recombination exist not only in lymphocytes but also in other types of cells from bacteria to humans where they help to increase adaptability of these organisms to adverse environmental conditions.[39,112] Recognized as molecular "engines of evolution", these mutagenic and recombination mechanisms are now considered targets for anti-evolvability therapy as illustrated by a drug, which by blocking ROS-induced hypermutation in *E. coli*, inhibits evolution of these microbes.[113–115] Thus, instead of being recent invention in the phylogeny, diversification mechanisms of lymphocytes represent some of the most fundamental systems, that lie at the heat of biological variability and adaptability.

The parallelism between variation generating mechanisms of lymphocytes and stress-induced "evolvability enhancers" in microbes and multicellular organisms suggests that these mechanisms have been co-opted, en masse, by the adaptive immune cells to permit evolvability of these cells. In this scenario, immune cells recruited ancient "evolvability cassettes" in the form of conserved genomic stress pathways to use them as responses to self-inflicted DNA damage (**Figure 5**). How stress-induced

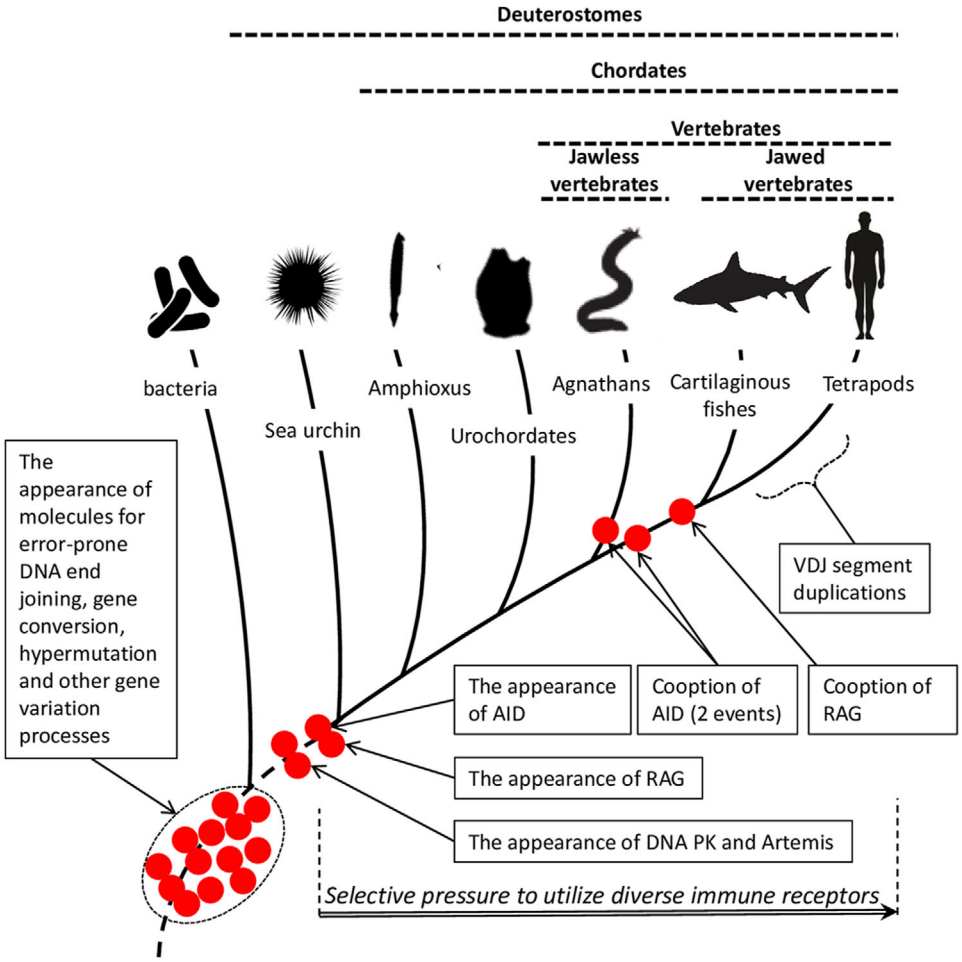

Figure 5. Evolution of lymphocyte evolvability by co-option of conserved variation-generating pathways. In this scenario, most components of the mutagenic DNA repair pathways were already present in unicellular precursors of metazoan species. DNA PK, Artemis and RAG enzymes appeared later in a common ancestor of deuterostomes. AID appeared only in ancestors of vertebrates but recent studies revealed that closely related members of AID/APOBEC already exist in echinoderms playing immune defense functions. Lymphocytes acquired the capacity to evolve somatically when they repurposed AID and RAG1/2 enzymes to target their own DNA while employing error-prone mutagenic and recombination pathways to repair the lesions. For RAG this co-option took place in the precursors of jawed vertebrate species, whereas for AID it happened twice: Once in jawless fishes and another time in jawed vertebrates. Later events were associated with the regulation and targeting of the DNA damaging enzymes to the antigen receptor gene loci as well as with gene duplication to generate multiple V(D)J segments.

diversification systems became repurposed to serve immune functions might be understood by analogy with a stress response system of *Arabidopsis*, which when exposed to infection stress increases frequency of somatic recombination, possibly affecting also resistance genes and thus mediating acquired resistance of the plant to the virus.[116] It is conceivable that SHM and V(D)J recombination evolved from such stress response systems, which initially operating on a larger scale, narrowed down later their mutagenic focus on immune receptors (Ig family members, leucine-rich repeat proteins, etc.). In the case of V(D)J recombination the co-option of a stress recombination system also required a shift from a reactive to an anticipative form, a transition conceivable as a switch from a "shotgun" immunity to Darwinian immunity.[117,118]

Critical for the process of co-option of stress-induced mutagenic systems was the adoption of AID and RAG enzymes to produce self-inflicted DNA lesions that would activate and direct stress response pathways to immune receptor genes.[119] Structural studies shed light on the events that led to domestication of RAG transposase, suggesting that association of RAG activity with the NHEJ pathway was a pivotal step towards transformation of this enzyme into a V(D)J recombinase.[120,121] Steps that led to the adoption of the AID are less clear but recent discovery of AID/APOBEC family members in echinoderms and brachiopods helps to elucidate these events.[30] They suggest that these enzymes initially emerged as mutators of foreign genes and that they acquired capacity to target endogenous genes only in vertebrates. The observation that genotoxic stress potentiates the activity of AID/APOBEC enzymes in mammals raises possibility that this mutagenic activity was developing as a form of stress response against these exogenous elements.[30,122] In addition to the co-option of stress induced mutagenic systems as well as AID and RAG enzymes, the emergence of a clonally evolving immune cell population also required co-option and reuse of ancient

cellular fate timers and other control systems to mediate coordination of replicative and fate-related decision-making following cell activation.[123] All in all, a general picture emerges in which co-option and reuse of ancient DNA processing genes, repair pathways and regulatory pathways allowed for the development of somatically evolvable adaptive immune cell populations.

Advances in our understanding of the agnathan immunity further support this framework suggesting that diversification mechanisms in jawless fish also developed from stress response system. In fact, despite utilizing leucin rich repeat (LRR)-based variable lymphocyte receptors (VLRs) rather than immunoglobulin sensors, jawless fish use some of the enzymes that mediate hypermutation of B cell receptor genes.[124] Lampreys employ two AID orthologs, CDA1, and CDA2, to produce DNA lesions in their lymphocytes and rely on a gene conversion-like mechanism (so called "copy choice") to generate VLR variation.[125–127] While the molecular mechanism of copy choice still awaits elucidation, a comparison with a diversification system in the yeast *Schizosaccharomyces pombe* already suggests an involvement of conserved variation generating pathways in this process.[125] It will be also of interest to understand whether and how "custom-fit" forms of immunity in invertebrates utilize elements of conserved, variation generating systems to generate immune receptor diversity.[128] All an all, accumulating data suggest that variation-generating mechanisms in lymphocytes predate emergence of antigen receptors and that their presence in the immune cells is a result of co-option of ancient stress response mechanisms that act as "evolvability enhancers" in unicellular species.

6. Conclusions and Outlook

Diversity is essential for survival and organisms have to balance stability and mutability to persist and evolve.[129] While oncosuppressors and other guardians of genomic integrity limit the capacity of somatic cells to diversify their genes, lymphocytes co-opted ancient mutagenic and gene recombination mechanisms to reclaim their primordial capacity to evolve and vary. Instead of co-opting ubiquitous DNA repair pathways and transforming them into variation generating mechanisms, lymphocyte precursors already co-opt specialized systems for diversity and evolvability. These observations call for reevaluation of the "big bang" view of immune evolution[130] and draw attention to the microbial DNA processing systems as likely precursors for adaptive immune variation mechanisms. They also highlight importance of repurposing and integration of pre-existing systems in the evolution of immunity.[131,132]

The likely co-option of variation generating systems by lymphocytes helps to shed light on the problem of evolution of evolvability in so far as it supports the view that the potential to evolve depends on acquisition of specialized variation generating systems acting as "engines of evolution" for the corresponding cell population.[112,117,133,134] Overall, integrated studies of adaptive immunity and stress-induced mutagenesis contribute to our understanding of the evolution of evolvability and suggest that programmed gene alterations are not secondary products of evolution but key processes that make evolution possible. This, in turn, challenges the Neo-Darwinian framework depicting evolution as reliant on genetic accidents rather than on regulated variation.

Conflict of Interest

The author declares no conflict of interest.

Keywords

evolvability, gene conversion, genomic stress, hypermutation, lymphocyte evolution, mutagenesis, recombination V(D)J

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[1] H. Chen, F. Lin, K. Xing, X. He, *Nat. Commun.* **2015**, *6*, 6367.
[2] A. S. Trigos, R. B. Pearson, A. T. Papenfuss, D. L. Goode, *Br. J. Cancer* **2018**, *118*, 145.
[3] P. Godfrey-Smith, *Darwinian Populations and Natural Selection*, Oxford University Press, New York **2009**.
[4] L. W. Buss, *The Evolution of Individuality*, Princeton University Press, Princeton, NJ **1987**.
[5] F. M. Burnet, *The Clonal Selection Theory of Acquired Immunity*, Vanderbilt University Press, Nashville, TN **1959**.
[6] A. I. Tauber, S. H. Podolsky, *The Generation of Diversity: Clonal Selection Theory and the Rise of Molecular Immunology*, Harvard University Press, Cambridge, MA **2000**.
[7] P. D. Hodgkin, W. R. Heath, A. G. Baxter, *Nat. Immunol.* **2007**, *8*, 1019.
[8] T. Boehm, *Nat. Rev. Immunol.* **2011**, *11*, 307.
[9] U. Hershberg, E. T. Luning Prak, *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **2015**, *370*, 20140239.
[10] I. R. Cohen, *Tending Adam's Garden: Evolving the Cognitive Immune Self*, Academic Press, New York **2000**.
[11] J. L. Payne, A. Wagner, *Nat. Rev. Genet.* **2019**, *20*, 24.
[12] M. Bahjat, J. E. J. Guikema, *Review Int. J. Mol. Sci.* **2017**, *18*, 1876.
[13] N. Y. Yao, M. E. O'Donnell, *Crit. Rev. Biochem. Mol. Biol.* **2016**, *51*, 135.
[14] M. F. Goodman, R. Woodgate, *Cold Spring Harb. Perspect. Biol.* **2013**, *5*, a010363.
[15] J. C. Weill, C. A. Reynaud, *Nat. Rev. Immunol.* **2008**, *8*, 302.
[16] J. Trincao, R. E. Johnson, C. R. Escalante, S. Prakash, L. Prakash, A. K. Aggarwal, *Mol. Cell* **2001**, *8*, 417.
[17] M. Diaz, L. K. Verkoczy, M. F. Flajnik, N. R. Klinman, *J. Immunol.* **2001**, *167*, 327.
[18] D. Schenten, S. Kracker, G. Esposito, S. Franco, U. Klein, M. Murphy, F. W. Alt, K. Rajewsky, *J. Exp. Med.* **2009**, *206*, 477.
[19] M. J. Martin, L. Blanco, in *Repair – New Research Directions* (Ed: C. Chen) InTech, Rijeka, Croatia **2013**, pp. 85–121.
[20] T. Hibino, M. Loza-Coll, C. Messier, A. J. Majeske, A. H. Cohen, D. P. Terwilliger, K. M. Buckley, V. Brockton, S. V. Nair, K. Berney, S. D. Fugmann, M. K. Anderson, Z. Pancer, R. A. Cameron, L. C. Smith, J. P. Rast, *Dev. Biol.* **2006**, *300*, 349.
[21] A. E. Clatworthy, M. A. Valencia, J. E. Haber, M. A. Oettinger, *Mol. Cell* **2003**, *12*, 489.
[22] A. J. Doherty, S. P. Jackson, G. R. Weller, *FEBS Lett.* **2001**, *500*, 186.
[23] Y. Aylon, M. Kupiec, *DNA Repair (Amst.)* **2004**, *3*, 797.
[24] K. Fukui, *J. Nucleic Acids* **2010**, *2010*, 260512.
[25] N. Schormann, R. Ricciardi, D. Chattopadhyay, *Protein Sci.* **2014**, *23*, 1667.
[26] B. Demple, L. Harrison, *Annu. Rev. Biochem.* **1994**, *63*, 915.
[27] B. Ribar, T. Izumi, S. Mitra, *Nucleic Acids Res.* **2004**, *32*, 115.
[28] D. Bonatto, L. F. Revers, M. Brendel, J. A. Henriques, *Braz. J. Med. Biol. Res.* **2005**, *38*, 321.

[29] S. G. Conticello, C. J. F. Thomas, S. K. Petersen-Mahrt, M. S. Neuberger, *Mol. Biol. Evol.* **2005**, *22*, 367.
[30] M. C. Liu, W. Y. Liao, K. M. Buckley, S. Y. Yang, J. P. Rast, S. D. Fugmann, *Nat. Commun.* **2018**, *9*, 1948.
[31] S. D. Fugmann, C. Messier, L. A. Novack, R. A. Cameron, J. P. Rast, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 3728.
[32] A. Krishnan, L. M. Iyer, S. J. Holland, T. Boehm, L. Aravind, *Proc. Natl. Acad. Sci. USA* **2018**, *115*, E3201.
[33] S. D. Fugmann, *Semin. Immunol.* **2010**, *22*, 10.
[34] C. Liu, Y. Yang, D. G. Schatz, *Nature* **2019**, *575*, 540.
[35] V. V. Kapitonov, E. V. Koonin, *Biol. Direct* **2015**, *10*, 20.
[36] L. M. Carmona, D. G. Schatz, *FEBS J.* **2017**, *284*, 1590.
[37] S. Huang, X. Tao, S. Yuan, Y. Zhang, P. Li, H. A. Beilinson, Y. Zhang, W. Yu, P. Pontarotti, H. Escriva, Y. Le Petillon, X. Liu, S. Chen, D. G. Schatz, A. Xu, *Cell* **2016**, *166*, 102.
[38] E. C. Martin, C. Vicari, L. Tsakou-Ngouafo, P. Pontarotti, A. J. Petrescu, D. G. Schatz, *Mob. DNA* **2020**, *11*, 17.
[39] D. M. Fitzgerald, P. J. Hastings, S. M. Rosenberg, *Annu. Rev. Cancer Biol.* **2017**, *1*, 119.
[40] D. M. Fitzgerald, S. M. Rosenberg, *PLoS Genet.* **2019**, *15*, e1007995.
[41] C. Shee, J. L. Gibson, M. C. Darrow, C. Gonzalez, S. M. Rosenberg, *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 13659.
[42] A. Gutierrez, L. Laureti, S. Crussard, H. Abida, A. Rodríguez-Rojas, J. Blázquez, Z. Baharoglu, D. Mazel, F. Darfeuille, J. Vogel, I. Matic, *Nat. Commun.* **2013**, *4*, 1610.
[43] H. C. Tsui, G. Feng, M. E. Winkler, *J. Bacteriol.* **1997**, *179*, 7476.
[44] H. H. Y. Chang, N. R. Pannunzio, N. Adachi, M. R. Lieber, *Nat. Rev. Mol. Cell Biol.* **2017**, *18*, 495.
[45] S. J. Quillin, H. S. Seifert, *Nat. Rev. Microbiol.* **2018**, *16*, 226.
[46] L. A. Cahoon, H. S. Seifert, *Mol. Microbiol.* **2011**, *81*, 1136.
[47] S. J. Norris, *Microbiol. Spectr.* **2014**, *2*, 1.
[48] E. Heidenreich, *Crit. Rev. Biochem. Mol. Biol.* **2007**, *42*, 285.
[49] E. Shor, C. A. Fox, J. R. Broach, *PLoS Genet.* **2013**, *9*, e1003680.
[50] R. S. Galhardo, P. J. Hastings, S. M. Rosenberg, *Crit. Rev. Biochem. Mol. Biol.* **2007**, *42*, 399.
[51] G. P. Rodriguez, N. V. Romanova, G. Bao, N. C. Rouf, Y. W. Kow, G. F. Crouse, *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 6153.
[52] N. Rajaei, K. K. Chiruvella, F. Lin, S. U. Aström, *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 15491.
[53] P. de Francisco, A. Martín-González, A. P. Turkewitz, J. C. Gutiérrez, *Environ. Microbiol.* **2018**, *20*, 2410.
[54] K. W. Deitsch, S. A. Lukehart, J. R. Stringer, *Nat. Rev. Microbiol.* **2009**, *7*, 493.
[55] C. M. Turner, *FEMS Microbiol. Lett.* **1997**, *153*, 227.
[56] F. J. J. Chain, J. M. Flynn, J. K. Bull, M. E. Cristescu, *Genome Res.* **2019**, *29*, 64.
[57] C. Matsuba, D. G. Ostrow, M. P. Salomon, A. Tolani, C. F. Baer, *Biol. Lett.* **2012**, *9*, 20120334.
[58] A. F. Agrawal, A. D. Wang, *PLoS Biol.* **2008**, *6*, e30.
[59] J. Yuan, L. Narayanan, S. Rockwell, P. M. Glazer, *Cancer Res.* **2000**, *60*, 4372.
[60] R. Kumareswaran, O. Ludkovski, A. Meng, J. Sykes, M. Pintilie, R. G. Bristow, *J. Cell Sci.* **2012**, *125*, 189.
[61] Y. Lu, A. Chu, M. S. Turker, P. M. Glazer, *Mol. Cell Biol.* **2011**, *31*, 3339.
[62] V. T. Mihaylova, R. S. Bindra, J. Yuan, D. Campisi, L. Narayanan, R. Jensen, F. Giordano, R. S. Johnson, S. Rockwell, P. M. Glazer, *Mol. Cell. Biol.* **2003**, *23*, 3265.
[63] M. Russo, G. Crisafulli, A. Sogari, N. M. Reilly, S. Arena, S. Lamba, A. Bartolini, V. Amodio, A. Magrì, L. Novara, I. Sarotto, Z. D. Nagel, C. G. Piett, A. Amatu, A. Sartore-Bianchi, S. Siena, A. Bertotti, L. Trusolino, M. Corigliano, M. Gherardi, M. C. Lagomarsino, F. Di Nicolantonio, A. Bardelli, *Science* **2019**, *366*, 1473.
[64] I. Martincorena, *Genome Med.* **2019**, *11*, 35.
[65] K. Naxerova, *Sci. Transl. Med.* **2019**, *11*, eaax4871.
[66] A. W. Duncan, A. E. H. Newell, W. Bi, M. J. Finegold, S. B. Olson, A. L. Beaudet, M. Grompe, *J. Clin. Invest.* **2012**, *122*, 3307.
[67] R. G. Hunter, K. Gagnidze, B. S. McEwen, D. W. Pfaff, *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 6828.
[68] P.-C. Wei, A. N. Chang, J. Kao, Z. Du, R. M. Meyers, F. W. Alt, B. Schwer, *Cell* **2016**, *164*, 644.
[69] A. I. Tauber, L. Chernyak, *Metchnikoff and the Origins of Immunology: From Metaphor to Theory*, Oxford University Press, Oxford, UK **1991**.
[70] E. Barsoum, P. Martinez, S. U. Aström, *Genes Dev.* **2010**, *24*, 33.
[71] M. Gellert, *Annu. Rev. Biochem.* **2002**, *71*, 101.
[72] X. Chen, Y. Cui, R. B. Best, H. Wang, Z. H. Zhou, W. Yang, M. Gellert, *Nat. Struct. Mol. Biol.* **2020**, *27*, 119.
[73] M. Lapkouski, W. Chuenchor, M. S. Kim, M. Gellert, W. Yang, *J. Biol. Chem.* **2015**, *290*, 14618.
[74] D. B. Roth, J. P. Menetski, P. B. Nakajima, M. J. Bosma, M. Gellertm, *Cell* **1992**, *70*, 983.
[75] D. G. Schatz, P. C. Swanson, *Annu. Rev. Genet.* **2011**, *45*, 167.
[76] V. V. Kapitonov, J. Jurka, *PLoS Biol.* **2005**, *3*, e181.
[77] K. K. Chiruvella, N. Rajaei, V. R. Jonna, A. Hofer, S. U. Åström, *Sci. Rep.* **2016**, *6*, 21671.
[78] D. Jangam, C. Feschotte, E. Betrán, *Trends Genet.* **2017**, *33*, 817.
[79] S. Malu, V. Malshetty, D. Francis, P. Cortes, *Immunol. Res.* **2012**, *54*, 233.
[80] Y. Ma, U. Pannicke, K. Schwarz, M. R. Lieber, *Cell* **2002**, *108*, 781.
[81] E. A. Motea, A. J. Berdis, *Biochim. Biophys. Acta* **2010**, *1804*, 1151.
[82] C. H. Emerson, A. A. Bertuch, *Biochem. Cell. Biol.* **2016**, *94*, 396.
[83] C. Bertrand, A. Thibessard, C. Bruand, F. Lecointe, P. Leblond, *Mol. Microbiol.* **2019**, *111*, 1139.
[84] P. Dupuy, L. Sauviac, C. Bruand, *Nucleic Acids Res.* **2019**, *47*, 1335.
[85] I. Corcoles-Saez, K. Dong, A. L. Johnson, E. Waskiewicz, M. Costanzo, C. Boone, R. S. Cha, *Dev. Cell* **2018**, *46*, 495.
[86] T. Swings, B. Van den Bergh, S. Wuyts, E. Oeyen, K. Voordeckers, K. J. Verstrepen, M. Fauvart, N. Verstraeten, J. Michiels, *Elife* **2017**, *6*, e22939.
[87] B. Pilzecker, H. Jacobs, *Front. Immunol.* **2019**, *10*, 438.
[88] M. F. Flajnik, *Nat. Rev. Immunol.* **2018**, *18*, 438.
[89] J. A. Ott, C. D. Castro, T. C. Deiss, Y. Ohta, M. F. Flajnik, M. F. Criscitiello, *Elife* **2018**, *7*, e28477.
[90] C. A. Schramm, D. C. Douek, *Front. Immunol.* **2018**, *9*, 1876.
[91] M. F. Goodman, *Environ. Mol. Mutagen.* **2016**, *57*, 421.
[92] B. Tippin, M. F. Goodman, *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **2001**, *356*, 47.
[93] D. F. Allison, G. G. Wang, *Cell Stress* **2019**, *3*, 38.
[94] R. Pavri, *Genes (Basel)* **2017**, *8*, 154.
[95] M. Samaranayake, J. M. Bujnicki, M. Carpenter, A. S. Bhagwat, *Chem. Rev.* **2006**, *106*, 700.
[96] E. S. Tang, A. Martin, *DNA Repair (Amst)* **2007**, *6*, 1557.
[97] C. Vink, G. Rudenko, H. S. Seifert, *FEMS Microbiol. Rev.* **2012**, *36*, 917.
[98] E. C. Ordinario, M. Yabuki, P. Handa, W. J. Cummings, N. Maizels, *BMC Mol. Biol.* **2009**, *10*, 98.
[99] S. A. Hill, J. K. Davies, *FEMS Microbiol. Rev.* **2009**, *33*, 521.
[100] K. Kurosawa, K. Ohta, *Genes (Basel)* **2011**, *2*, 48.
[101] N. Maizels, L. Davis, *Nucleic Acids Res.* **2018**, *46*, 6962.
[102] L. L. Prister, J. Xu, H. S. Seifert, *J. Bacteriol.* **2019**, *201*, e00256.
[103] L. A. Cahoon, H. S. Seifert, *Science* **2009**, *325*, 764.
[104] Q. Qiao, L. Wang, F. L. Meng, J. K. Hwang, F. W. Alt, H. Wu, *Mol. Cell* **2017**, *67*, 361.
[105] L. Glover, S. Hutchinson, S. Alsford, D. Horn, *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 7225.
[106] P. Borst, *Cell* **2002**, *109*, 5.

[107] F. Aresta-Branco, E. Erben, F. N. Papavasiliou, C. E. Stebbins, *Trends Parasitol.* **2019**, *35*, 302.
[108] N. Maizels, *Annu. Rev. Genet.* **2005**, *39*, 23.
[109] C. Shee, J. L. Gibson, S. M. Rosenberg, *Cell Rep.* **2012**, *2*, 714.
[110] H. Wimberly, C. Shee, P. C. Thornton, P. Sivaramakrishnan, S. M. Rosenberg, P. J. Hastings, *Nat. Commun.* **2013**, *4*, 2115.
[111] S. J. Hanson, K. H. Wolfe, *Genetics* **2017**, *206*, 9.
[112] J. A. Shapiro, *Evolution: A View from the 21st Century*, FT Press, Upper Saddle River, NJ **2011**.
[113] J. P. Pribis, L. García-Villada, Y. Zhai, O. Lewin-Epstein, A. Z. Wang, J. Liu, J. Xia, Q. Mei, D. M. Fitzgerald, J. Bos, R. H. Austin, C. Herman, D. Bates, L. Hadany, P. J. Hastings, S. M. Rosenberg, *Mol. Cell* **2019**, *74*, 785.
[114] S. M. Rosenberg, C. Queitsch, *Science* **2014**, *343*, 1088.
[115] A. Cipponi, D. M. Thomas, *BioEssays* **2014**, *36*, 552.
[116] I. Kovalchuk, O. Kovalchuk, V. Kalck, V. Boyko, J. Filkowski, M. Heinlein, B. Hohn, *Nature* **2003**, *423*, 760.
[117] E. Muraille, *Front. Microbiol.* **2018**, *9*, 223.
[118] V. Müller, R. J. de Boer, S. Bonhoeffer, E. Szathmáry, *Biol. Rev. Camb. Philos. Soc.* **2018**, *93*, 505.
[119] A. Tubbs, A. Nussenzweig, *Cell* **2017**, *168*, 644.
[120] Y. Zhang, T. C. Cheng, G. Huang, Q. Lu, M. D. Surleac, J. D. Mandell, P. Pontarotti, A. J. Petrescu, A. Xu, Y. Xiong, D. G. Schatz, *Nature* **2019**, *569*, 79.
[121] M. R. Lieber, *Trends Immunol.* **2019**, *40*, 668.
[122] B. Mussil, R. Suspène, V. Caval, A. Durandy, S. Wain-Hobson, J.-P. Vartanian, *Sci. Rep.* **2019**, *9*, 3109.
[123] M. Horton, E. Hawkins, S. Heinzel, P. Hodgkin, *Immunol. Cell Biol.* **2020**, *98*, 439.
[124] M. F. Flajnik, M. Kasahara, *Nat. Rev. Genet.* **2010**, *11*, 47.
[125] F. Nagawa, N. Kishishita, K. Shimizu, S. Hirose, M. Miyoshi, J. Nezu, T. Nishimura, H. Nishizumi, Y. Takahashi, S. Hashimoto, M. Takeuchi, A. Miyajima, T. Takemori, A. J. Otsuka, H. Sakano, *Nat. Immunol.* **2007**, *8*, 206.
[126] T. Boehm, M. Hirano, S. J. Holland, S. Das, M. Schorpp, M. D. Cooper, *Annu. Rev. Immunol.* **2018**, *36*, 19.
[127] I. B. Rogozin, L. M. Iyer, L. Liang, G. V. Glazko, V. G. Liston, Y. I. Pavlov, L. Aravind, Z. Pancer, *Nat. Immunol.* **2007**, *8*, 647.
[128] J. Rimer, I. R. Cohen, N. Friedman, *BioEssays* **2014**, *36*, 273.
[129] I. Martincorena, N. M. Luscombe, *BioEssays* **2013**, *35*, 123.
[130] M. F. Flajnik, *Curr. Biol.* **2014**, *24*, R1060.
[131] G. W. Litman, J. P. Rast, S. D. Fugmann, *Nat. Rev. Immunol.* **2010**, *10*, 543.
[132] R. S. Harris, Q. Kong, N. Maizels, *Mutat. Res.* **1999**, *436*, 157.
[133] R. Hershberg, *Cold Spring Harb. Perspect. Biol.* **2015**, *7*, a018077.
[134] E. V. Koonin, Y. I. Wolf, *Front. Cell. Infect. Microbiol.* **2012**, *2*, 119.