Available online at www.sciencedirect.com
SCIENCE DIRECT®
Research in Microbiology 155 (2004) 360–369
Research in Microbiology
www.elsevier.com/locate/resmic

Adaptation to the deleterious effects of antimicrobial drug resistance mutations by compensatory evolution

Sophie Maisnier-Patin [a,b], Dan I. Andersson [a,b,*]

[a] *Swedish Institute for Infectious Disease Control, Department of Bacteriology, 171 82 Solna, Sweden*
[b] *Karolinska Institute, Microbiology and Tumour Biology Center, 171 77 Stockholm, Sweden*

Received 5 September 2003; accepted 20 January 2004

Available online 12 April 2004

This review is dedicated to the memory of Michel Blot

Abstract

Compensatory mutations, due to their ability to mask the deleterious effects of another mutation, are important for the adaptation and evolution of most organisms. Resistance to antibiotics, antivirals, antifungals, herbicides and insecticides is usually associated with a fitness cost. As a result of compensatory evolution, the initial fitness costs conferred by resistance mutations (or other deleterious mutations) can often be rapidly and efficiently reduced. Such compensatory evolution is potentially of importance for (i) the long-term persistence of drug resistance, (ii) reducing the rate of fitness loss associated with the accumulation of deleterious mutations in small asexual populations, and (iii) the evolution of complexity of cellular processes.

Keywords: Antibiotic resistance; Deleterious mutation; Fitness cost; Compensatory mutation; Adaptation

1. Introduction

Most mutations that occur in any organism are deleterious. A deleterious mutation has several different potential fates; it may go extinct, persist at some frequency in the population, revert back to the parental wild-type state or be compensated for by another second-site mutation (compensatory/suppressor mutation) that reduces its deleterious effect. Compensatory mutations (CMs) have been defined in several ways. For example, Wright [116,117] and Kimura [54] defined CMs as mutations that mask the deleterious effect of another mutation or as mutations that are independently deleterious but neutral when combined. CMs have been discussed in a variety of evolutionary contexts, such as, for example, the evolution of sex [58,112], the structure of fitness landscapes [18,89,109], fitness epistasis [80,93,107,112], mutational load and the extinction of small populations [36,61,80,93,112,113]. Similarly, geneticists have for a long time been involved in identifying "suppressor" mutations, and the identification and use of such mutations have proven to be an invaluable tool (for a review see [46]). Thus, they have allowed geneticists to identify structural, functional and regulatory interactions within and between proteins and/or RNA as well as between various biological processes.

Certain bacterial populations have probably been exposed to antibiotics for a long time but it is only within the last 60 years, since humans have been using antibiotics to treat infectious diseases, that the selection pressure has become particularly strong. As a result of this recent extensive use and misuse of antibiotics the frequency of resistance for most combinations of antibiotics and bacterial species has increased. Similar resistance development has also been observed in viruses, fungi, plants and insects towards respectively, antiviral drugs, antifungals, herbicides and insecticides, creating increasing medical and economic problems. Resistance to antibiotics, and drugs in general, occurs either by genomic mutations or by acquisition of horizontally transferred genetic material carrying resistance determinants. These genetic changes often have deleterious effects on the organism since they impair vital functions or confer metabolic burdens. This results in decreased fit-

* Corresponding author.
E-mail address: Dan.Andersson@smi.ki.se (D.I. Andersson).

0923-2508/$ – see front matter
doi:10.1016/j.resmic.2004.01.019

ness, which may be expressed as decreased survival, reduced growth rate, or for pathogenic organisms, as reduced transmission rate and virulence (reviewed in [1,3,64]). When the drug-selective pressure is removed, the resistant organisms with lower fitness are at a disadvantage compared to the parental susceptible ones. This means that the deleterious mutation may go extinct. Alternatively, it may revert back to its ancestral state or be compensated for by additional mutations. Compensation is of special interest since resistant organisms, while adapting genetically to the costs, may still maintain their resistance, resulting in the stabilization of resistant organisms in the population. In this review, we present a few selected examples of how and to what extent CMs can restore the loss of cellular or viral functions and indicate the importance of compensatory evolution in clinical situations involving bacteria and viruses resistant to antimicrobial drugs. Compensatory adaptation to deleterious mutations is also briefly discussed from a more general evolutionary perspective.

2. Types of compensatory mutations

Compensation can occur via several different mechanisms (Fig. 1). The first and perhaps the most common mechanism is the restoration of the structure and function of an altered RNA or protein by intragenic mutations. Second, intergenic mutations can restore the structure and function to a mutated multi-subunit complex molecule or organelle such as RNA polymerase or the ribosome. A third compen-

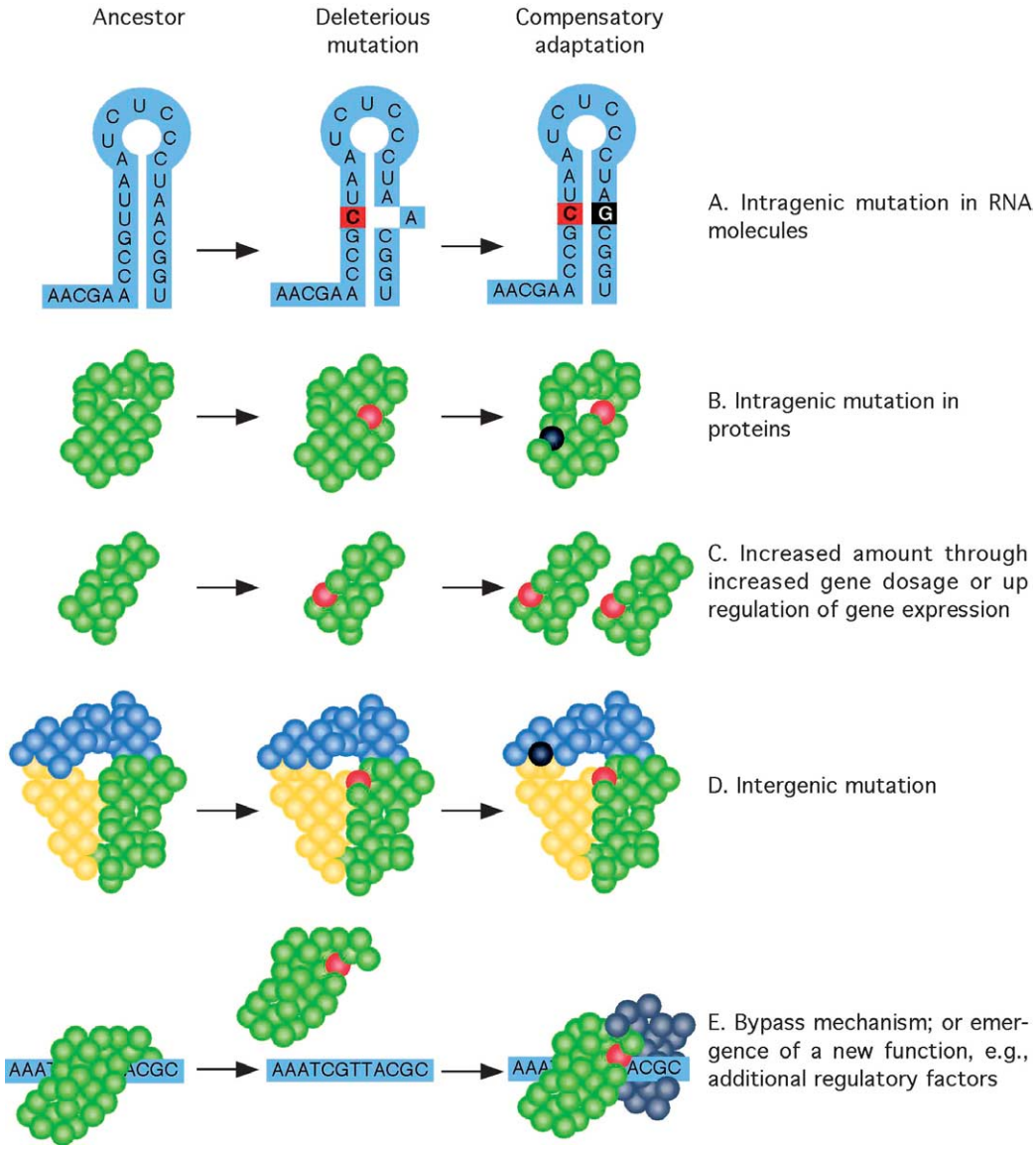

Fig. 1. Types of compensatory mechanisms. Restoration of the structure and function of an altered (A) RNA or (B) protein by intragenic mutations, (C) function amplification, (D) intergenic mutations in a multi-subunit molecule, or (E) by a bypass mechanism in which the mutated function is substituted by an alternative pathway or factor. Deleterious mutations are indicated with red spheres and compensatory mutations are symbolized in black.

sation mechanism is one in which the demand for the mutated function is reduced, for example, by a bypass mechanism wherein the mutated function is replaced with an alternative pathway. Finally, a defect enzyme can be compensated for simply by an increased amount of enzyme. For an interesting example of such compensation see the review by Roth and Andersson in the current issue.

The compensation process is commonly followed experimentally by serially passaging the low-fitness resistant strains in laboratory media, either in chemostats or in batch cultures, in cultured cell lines or in experimental animals. Using these procedures, faster-growing or more virulent mutants are selected in the absence or presence of the drug. Subsequently, the extent of compensation can be measured by quantifying a fitness parameter (e.g., infectivity, growth rate etc). Often this is done by pair-wise competition of the compensated mutant with the ancestral resistant mutant or with a reference strain that is genetically tagged. CMs can restore fitness to various degrees, sometimes to the ancestral or wild-type level. To assess the fitness-increasing specificity of CMs, several control experiments might be performed. First, the parental drug-susceptible strain can be evolved under similar conditions. If the putative CMs are not found in the evolved drug-susceptible background, it is likely that the identified CMs are not generally increasing fitness but rather specific suppressors of the resistance mutation. Second, the CMs may be transferred to a susceptible genetic background where they should not increase fitness if they are specific CMs.

2.1. Intragenic compensation to restore an altered function or structure

A classic example of intragenic compensation is provided by the genetic experiments carried out in 1961 using *rII* mutants of bacteriophage T4 defective in growth to show that some phenotypic revertants could reestablish infection of *Escherichia coli* K12. Analysis of these revertants revealed that they were still carrying the original frameshift mutation in *rII* along with a second suppressor mutation in the same gene. These analyses served to ascertain the three-letter organization of the genetic code [23]. Compensation by additional mutations in the mutated gene to restore the original structure or function of the gene product has since been widely observed in many organisms. By modeling the energetic folding of the mutant proteins or analysis of their X-ray structure, it has been shown that CMs often improve protein activity by restoring a stable conformation [4,17,45,53,77, 86,115].

Most of the deleterious resistance mutations in the human immunodeficiency virus type 1 (HIV-1) that reduce the ability of the virus to invade host cells and propagate can be compensated for by intragenic CMs, restoring the levels of viral replication to, or close to, the wild-type level [22,75, 85,106]. After 12 weeks of propagation in T cells, fitness of HIV-1 carrying the amino acid substitution P109S in the integrase could be restored by the compensatory substitution T125A [106]. These two mutations are located far apart on the integrase but both are part of the dimer interface of the enzyme. While P109S abolishes the 3′-end processing and DNA transfer activity of the HIV-1 integrase, the function of CM might be to restabilize the protein-protein interaction disrupted by the P109S substitution, and thus, to reestablish the integrase catalytic activity and viral infectivity. Viruses resistant to nucleoside analogues and non-nucleoside reverse transcriptase inhibitors have acquired mutations in the reverse transcriptase, which result in a lowered affinity for the natural deoxynucleoside triphosphates and for the template-primer substrate leading to reduced primer extension activity and viral replication [101]. Resistance mutations located near the active site of the reverse transcriptase usually change the conformation of the nucleotide binding site so that the entry of the nucleoside analogues or inhibitors is sterically inhibited. Prolonged exposure of the viruses to the drugs lead to compensatory substitutions in the amino acids located in the domain of the reverse transcriptase that interacts with the template-primer substrate [16,56,84,88]. These CMs may change the reverse transcriptase conformation to increase its catalytic efficiency by facilitating the entry of natural deoxynucleotides into the enzymatic active site and its interaction with the template-primer substrate.

Reduction of the biological cost of resistance by intragenic compensation has been shown to occur in bacteria resistant to fusidic acid [51,83], streptomycin [8,9, 74,102], β-lactams [50,91,104], rifampicin [8,96], sulfonamides [38] and coumarins [11]. Fusidic-acid-resistant mutants carry amino acid substitutions in elongation factor G. This results in a defect in GTP binding, a concomitant decrease in peptidyl-tRNA movement during the elongation phase of translation and slowed bacterial growth. A specific resistant mutant of *Salmonella typhimurium* could acquire at least 18 different CMs in EF-G that partly or fully restored the growth rate. Many of the CMs probably facilitated the transition of EF-G from the GDP- to the GTP-binding state [51]. Similarly, a streptomycin resistance mutation in the *rpsL* gene (encoding ribosomal protein S12) concomitantly increases translational accuracy and decreases the elongation rate, resulting in an impairment of bacterial growth. The fitness costs of the restrictive mutation N42K could be intragenically compensated for by the mutation R93H (Fig. 2B) [74]. This residue is in contact with 16S RNA at the translation initiation site, and the substitution R93H is likely to disrupt interactions with the phosphates at positions 910 and 911. It may thereby destabilize the restrictive conformation and restore the wild-type translational accuracy and elongation rate. Resistance to the coumarin drugs, which bind to GyrB, one of the two subunits of DNA gyrase, is due to the substitution of the two amino acids R136 and G164 in the drug and ATP binding site. These mutations inhibit ATP hydrolysis and consequently reduce DNA supercoiling and bacterial growth. The resistance mutations introduce a tighter constraint on the loop region in

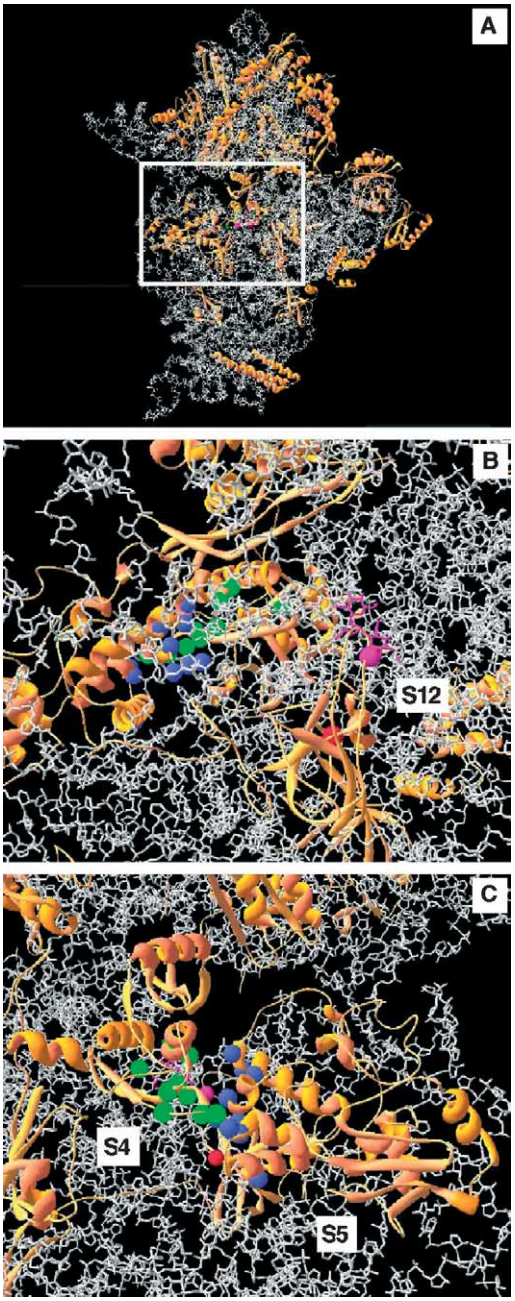

Fig. 2. Position in the 30 S ribosome subunit of *T. thermophilus* (A) (PDB accession number 1FJF) of a streptomycin resistance mutation and fitness-compensating mutations (B and C) isolated in *S. typhimurium*. The mutation (K42N) in the ribosomal protein S12 that confers streptomycin resistance is indicated with a pink sphere, the CMs in S4, S5 and S12 by blue, green and red spheres, respectively (adapted from [74]). Ribosomal proteins are shown in brown and 16S RNA in gray.

the catalytic site of the GyrB subunit, which can be relieved by two second-site mutations, H38Q or T157I, located at either end of a turn above the drug binding site [11]. The substitution T157I is thought to give a more favorable hydrophobic interaction that helps to stabilize the loop. Finally, mutant β-lactamases with amino acid substitutions have enlarged binding sites that can hold the bulky oxyimino side-chains of the third generation cephalosporins and inactivate them [91,110]. In enlarging their catalytic site and extending substrate specificity, the β-lactamases became less active because of the loss of interactions that contribute to protein stability. The CM M182T located 17 Å from the active site in the hinge region between the two domains of the β-lactamase increases enzyme activity, possibly by stabilizing the protein structure near the active site [50,91,104].

There are many examples of compensatory evolution occurring in RNA molecules. The maintenance of helices is important in stabilizing RNAs against degradation and modification, as well as providing specific functional structures. Individual mutations occurring at nucleotides involved in pairing within functional RNA stem structures are typically deleterious if they destabilize a stem. CMs that reestablish the pairing or recreate the same RNA folding as the wild type have been observed to restore function of tRNAs [48], rRNAs [28,44,99], mRNAs [55,92,105] and RNA regulatory sequences [6,57,90].

Intragenic compensation may occur as well by emergence of a new function through gene duplication at the same locus. Gene amplification might confer compensation by increasing the amount of a limiting enzymatic activity (reviewed in [98], Roth and Andersson (2004) this issue). Homologous recombination between a calmodulin–neomycin-resistance fusion gene and the *Trypanosoma brucei* chromosome anywhere in the four tandem genomic calmodulin genes resulted in a recombined locus consisting of the chimeric neomycin resistance gene and four to zero functional calmodulin genes [30]. In the latter case, cells having only half of their normal number of intact calmodulin genes had a fitness cost. When evolved, these mutants acquired additional calmodulin genes, frequently by amplifying a calmodulin gene, and concomitantly reverted to normal growth rate. Compensatory gene amplification was also observed in the mosquito *Culex pipiens* resistant to organophosphate insecticides. The resistant mosquitos express a defective acetylcholinesterase, which results in malfunction of the cholinergic synapses of the central nervous system. The fitness costs in the resistant insects were lowered by duplication of the acetylcholinesterase gene. One copy was still conferring the resistance phenotype while an additional copy might provide wild-type acetylcholinesterase activity to improve fitness [63,95].

2.2. Intergenic compensations to restore altered function or structure

The identification of intergenic (extragenic) CMs has been particularly important in understanding the processes and components involved in protein synthesis. The first type of stop codon suppressor mutations discovered in the late 1950s were found in mutated aminoacyl-tRNA synthetases, which charged tRNAs with the non-cognate amino acid. Other suppressor mutations were located in tRNA genes and these mutations allowed the mutant tRNA to mistranslate

a stop codon as a sense codon, resulting in incorporation of an amino acid and production of a full-length protein (reviewed in [46,82]). Moreover, mutations in certain ribosomal proteins (e.g., S4 and S5) result in decreased accuracy of the translational decoding process (reviewed in [59]). Conversely, other ribosomal mutations (e.g., in protein S12) can lead to increased accuracy of protein synthesis. The increased accuracy of these mutants is associated with a reduced translation elongation rate and growth rate. The biological costs associated with the specific mutation K42N in S12 of *E. coli* and *S. typhimurium* conferring streptomycin resistance could be compensated for by extragenic second-site mutations in the ribosomal proteins S4, S5 or L19 [8,74,102]. In most of the compensatory mutants, fitness was partially restored and only a few mutants were similar to the streptomycin susceptible wild-type bacteria. The ribosomal CMs increased the rate of translation to wild-type or near wild-type levels [10,100]. In total, thirty-four different amino acid substitutions have been identified and at specific locations, several unrelated amino acids could substitute for the wild-type amino acid [74]. The compensatory changes in S4 and S5 map to, or close to, the interface between the two proteins (Fig. 2C). They may thus disrupt interactions between the two proteins or between the proteins and 16S RNA and destabilize the restrictive conformation of the ribosome.

An example of intergenic CMs that restore proper RNA-protein binding and functioning is provided in HIV-1. Here, a 16 nucleotides deletion disrupting the stem-loop structure downstream from the long terminal repeat (LTR) impaired viral RNA packaging. By serial passage of the mutant viruses, fitter variants that harbored additional point mutations located in the 14 amino acid p2 spacer peptide within Gag between the capsid and the nucleocapsid (NC7) proteins and in the NC7 protein were selected. The CM in NC7 is located in the zinc finger motif of the protein, which interacts with the RNA elements required for efficient viral assembly and infectiousness [68].

2.3. Compensation by reducing the need for an altered function or by formation of a bypass pathway

The accumulation of a non-metabolized substrate due to the decreased activity of a mutant enzyme or the lack of a metabolized substrate may be compensated for by mutations that force cells to use an alternate pathway, block the entry into the impaired pathway, reduce the need for the affected function or by substituting the affected function with an alternative function. For example, fitness of a bacteriophage T7 mutant carrying a deletion of its ligase gene was recovered by CMs in other genes that are involved in DNA metabolism such as viral endonuclease, helicase and DNA polymerase [97]. These mutations are likely to decrease the demand for a functional ligase. Decreased fitness of isoniazid-resistant *Mycobacterium tuberculosis* containing a mutation in *katG* is due to reduced catalase–peroxidase activity. Compensation by a promoter-up mutation in the *ahpC* gene encoding the alkyl hydroperoxidase could substitute for the lowered KatG catalase–peroxidase activity [103]. Overproduction of AhpC might thus partially restore the defect in the oxidative stress response caused by inactivation of the KatG catalase. Finally, substitution G137D in the carboxylesterase (*Rop-1* allele) of the Australian sheep blowfly *Lucilia cuprina* confers resistance to diazinon, an organophosphorous insecticide, and concomitantly perturbs the development of blowflies [26]. To restore fitness, the resistant flies have acquired a CM in the scalloped wing gene *Scl*, a homologue of the *Drosophila melanogaster Notch* gene involved in the determination of cell fate throughout development in a variety of tissues [26,78]. Although the precise function of each gene product is not known, it seems that gene interactions within or between pathways occur to restore fitness.

2.4. Cases in which restoration of fitness only occurs by stepwise or direct reversion

In a few cases it appears that fitness can only be regained by reversion. For example, the simian immunodeficiency virus model of AIDS with a deletion in the *nef* gene shows lower replication capacity. This deletion could be repaired in vivo by duplication and subsequent base changes until the amino acid sequence become identical to that of the virulent wild-type virus [111]. These changes occurred in parallel with reversion to virulence. Reversion also appeared to be the only mechanism for restoring fitness to a capsid-defective bacteriophage phiX174. When the virus was evolved in an *S. typhimurium* host, the virus lost its ability to grow on *E. coli* because of two to three substitutions in the major capsid gene that altered the virus attachment efficiency. Fitness (ability to grow on *E. coli*) was recovered after serial passages and occurred only by reversion at these same sites, rather than by second-site CMs [24].

3. Compensation versus reversion

Whether reversion or compensation is more likely to occur when a low-fitness drug-resistant mutant is allowed to evolve will depend on the mutation rates to compensation and reversion, the population size, the environment under which compensation occurs and the fitness of the compensated mutants as compared to the revertants. It has been shown that CMs are often far more frequent than reversion in reducing the deleterious effect of a mutation [12,18,35,51,74,80,96]. For example, the fitness cost associated with the amino acid substitution K42N in the ribosomal protein S12 could be compensated for by 35 different amino acid substitutions, located in S12 itself or in the ribosomal proteins S4, S5 or L19. From the multiple recovery frequency of CMs it was estimated that about $2/3$ of all possible changes had

been identified, implying that the total number of CMs is about 50 [74]. A large compensatory target is not specific to multi-protein complexes such as the ribosome. The loss of fitness in fusidic acid-resistant mutants of *S. typhimurium* can be compensated for by about 20 different amino acid substitutions in the EF-G protein [51]. Multiple CMs can also restore the fitness of defective viruses [14,97].

The higher number of possible CMs than back mutations (reversion) and the bottlenecks associated with serial passage result in compensatory evolution being more common than reversion [67]. Thus, the common compensated mutants are more likely than rare revertants to successfully pass through the bottlenecks associated with serial passages. There are, however, exceptions where deleterious mutations more often revert back to wild type instead of being compensated for by additional mutations (see previous paragraph). Also, in the clinical context, reversion rather than compensation has been observed in few cases. For low-fitness, fusidic acid resistant *S. typhimurium* and *S. aureus* true revertants rather than compensated mutants were selected in mice and on human skin, most likely because the revertants had complete restoration of fitness whereas the compensated mutants still showed disturbed growth [9,43]. An inability to produce appropriate levels of RpoS, an important regulator of expression of virulence factors in *S. typhimurium*, could explain the low fitness of the compensated mutants [72,73].

4. Importance of compensatory mutations for drug resistance development in clinical settings

The emergence of drug-resistant HIV-1 in patients receiving protease inhibitors is associated with a sequential accumulation of amino acid substitutions within the active site that make the enzyme resistant to the inhibitor. The reduced protease catalytic activity, and the concomitant decreased viral replication rate can be restored by additional mutations in the protease itself [85], the *gag* p1/p6 and p7/p1 protease cleavage sites [75,120] and in the Gag protein at non-cleavage sites [41]. As with protease inhibitors, compensation was observed in viruses that evolved in patients exposed to reverse transcriptase inhibitors (nucleoside analogues and non-nucleoside types). In several of these cases it has been shown by analysis of viruses isolated from patients and genetic reconstruction experiments that intragenic mutations in the reverse transcriptase could restore fitness to the resistant reverse transcriptase mutants [16,49,56,84].

As for viruses, reconstructed mutants carrying different combinations of mutations revealed the evolution pattern of resistant bacteria in clinical settings. For example, fitness compensation by upregulation of the *ahpC* gene in bacteria isolated from patients has been suggested to occur in *M. tuberculosis* that are resistant to isoniazid due to loss-of-function mutations in the *katG* gene (see Section 2.3) [103]. Mutations in the enzyme dihydropteroate synthase, which catalyzes the reaction leading to the formation of dihydropteroic, a precursor to folic acid, render the bacteria resistant to sulfonamides. *Neisseria meningiditis* isolated from patients with meningococcal disease and resistant to sulfonamides bore several mutations within the dihydropteroate synthase. The two substitutions F31L and G194C conferred antibiotic resistance while the P84S mutation compensated for the negative effect of the two resistance mutations [38]. Similarly, clinical isolates of *Streptococcus pneumoniae* resistant to trimethoprim carried multiple amino acids substitutions in the dihydrofolate reductase enzyme [76]. Resistance was conferred by the single amino acid substitution I100L in the trimethoprim binding site. Additional mutations in the C-terminal region of the dihydrofolate reductase compensated for the lower affinity of the enzyme for its substrate dihydrofolic acid generated by I100L. In addition, other mutations in the N-terminal part of the enzyme were shown to be associated with increased resistance to trimethoprim. Another example of compensatory evolution is the M182T substitution that is frequently found in β-lactam-resistant β-lactamases. This mutation possibly suppresses the folding and stability defects of the mutated resistant enzyme (see Section 2.1) [50,104].

Furthermore, a combination of substitutions (resistance and compensatory) in RNA polymerase, similar to that observed in *E. coli* in vitro [96], was detected in clinical rifampicin-resistant isolates of *M. tuberculosis* [94]. Three out of five fusidic acid-resistant strains of *Staphylococcus aureus* isolated from the blood of patients treated with fusidic acid had also acquired multiple mutations in the *fusA* gene similar to those identified in vitro that cause resistance and fitness compensation [83].

Finally, patient isolates of *Helicobacter pylori* that had developed resistance to clarithromycin during treatment had also acquired CMs [7]. By examining clonally related pairs of susceptible pre-treatment and resistant post-treatment strains of *H. pylori* it was shown that the cost of clarithromycin resistance was compensated for after only 3 months of growth in the stomach in two out of three patients examined. A similar approach was also used by Gillespie et al. [42] to study how fitness of multi-drug-resistant *M. tuberculosis* changed as the bacteria were transmitted from one patient to another during a hospital outbreak.

5. Compensatory mutations as a mechanism to reduce the rate of Muller's ratchet

Asexually reproducing organisms with small population sizes will, over time, accumulate deleterious mutations that ultimately can result in mutational meltdown and organism extinction [19,37,60,69–71,81]. Such a decrease in fitness due to repeated bottlenecks and stochastic samplings has been demonstrated experimentally with populations of bacteriophage ϕ6 [20], vesicular stomatis virus [29,32], foot-and-mouth disease virus [33], RNA bacteriophage MS2 [27], HIV-1 [118], bacteria [2,40,52] yeasts [119], proto-

zoa [5] and multicellular organisms [39,108]. The rate of this process (Muller's ratchet) can be reduced, for example, by sexual reproduction where the mutation-free class is restored by recombination, by mutational reversion to the mutation-free state or by the presence of large populations that allow the continued presence of the mutation-free class. Another potential mechanism might be compensatory evolution. Thus, as discussed above, there is compelling evidence that CMs are far more frequent than reversion in recovering fitness loss due to deleterious mutations. In addition, it has been demonstrated that by passaging large populations of fitness-impaired mutants substantial fitness gains occur by compensatory evolution in populations of viruses [18,21,31,34,68,87], bacteria [8,67,74,80] and multicellular organisms [36]. Furthermore, more recent theoretical models of fitness evolution also suggest that CMs might, at least under certain circumstances, provide a possible molecular mechanism to slow down the decay in fitness [61,62,93,109,112,114].

6. Compensatory evolution as a mechanism to genetically fix deleterious mutations/genetic elements and generate complexity

When separated from the initial deleterious mutation, CMs alone often reduce fitness [10,17,49,74,97]. This is also observed during evolution to reduce the costs of extrachromosomal elements, such as plasmids [15,65,79]. Coevolution between a bacterium and a plasmid resulted in a reduction in the deleterious effects of plasmid carriage, usually by CMs occurring on the host chromosome [13,25,47,66]. When the plasmid was removed from the evolved strain the plasmid-free evolved strain had reduced fitness as compared to the parental plasmid-free strain. The above results suggest that drug-susceptible revertants are unlikely to ascend because they are at a disadvantage in the genetic background of the CM. As a consequence, the organism will be constrained from reverting back to the ancestral mutation-free state. More generally, such fitness-reducing effects of the CMs themselves may provide a mechanism by which initially deleterious novel mutations or functions (e.g., plasmids, transposons) can become genetically fixed during evolution. An extension of this idea is the hypothesis that compensatory evolution can generate complexity in biological systems [121,122]. Thus, it has been proposed that weakly deleterious mutations, which result in loss of, for example, a gene regulatory protein, can be compensated for by the recruitment of an additional novel regulatory factor. A combination of accumulation of deleterious mutations and the resulting selection of CMs to restore a function could result in increased specificity and a higher level of interactions in any regulatory, metabolic or structural system. Thus, successive deleterious mutations and compensatory changes could constitute a major pathway for increasing the complexity of cellular processes and functions.

7. Conclusion

Several important conclusions emerge from the presented experimental and theoretical studies. First, it is clear that compensatory evolution is a very common and pervasive process, which has been observed in many viruses, bacteria, protozoa, yeast and multicellular organisms carrying deleterious mutations. Most types of deleterious mutations can be compensated for to some extent and generally a specific deleterious mutation can be suppressed by a surprisingly large variety of CMs. As a result the compensatory process is often rapid. With regard to drug resistance development, the available data suggest that compensatory evolution may cause stabilization of drug resistance in microorganisms. An unwanted medical consequence of this evolution is that reduced use of drugs might not result in lowered frequency of drug resistance. Finally, CMs could be important for slowing down the rate of fitness loss associated with the accumulation of deleterious mutations in small (asexual) populations, as well as providing a potential mechanism for increasing the complexity of cellular processes.

Acknowledgements

This work was supported by grants to D.I.A. from the Swedish Research Council, the Swedish Institute for Infectious Disease Control, the EU 5th Framework Program, the Swedish Strategic Research Foundation and the AFA Research Fund.

References

[1] D.I. Andersson, Persistence of antibiotic resistant bacteria, Curr. Opin. Microbiol. 6 (2003) 452–456.
[2] D.I. Andersson, D. Hughes, Muller's ratchet decreases fitness of a DNA-based microbe, Proc. Natl. Acad. Sci. USA 93 (1996) 906–907.
[3] D.I. Andersson, B.R. Levin, The biological cost of antibiotic resistance, Curr. Opin. Microbiol. 2 (1999) 489–493.
[4] L.A. Aramli, C.M. Teschke, Single amino acid substitutions globally suppress the folding defects of temperature-sensitive folding mutants of phage P22 coat protein, J. Biol. Chem. 274 (1999) 22217–22224.
[5] G. Bell (Ed.), Sex and Death in the Protozoa, Cambridge Univ. Press, New York, 1988.
[6] B. Berkhout, B. Klaver, A.T. Das, Forced evolution of a regulatory RNA helix in the HIV-1 genome, Nucleic Acids Res. 25 (1997) 940–947.
[7] B. Björkholm, M. Sjolund, P.G. Falk, O.G. Berg, L. Engstrand, D.I. Andersson, Mutation frequency and biological cost of antibiotic resistance in *Helicobacter pylori*, Proc. Natl. Acad. Sci. USA 98 (2001) 14607–14612.
[8] J. Björkman, D. Hughes, D.I. Andersson, Virulence of antibiotic-resistant *Salmonella typhimurium*, Proc. Natl. Acad. Sci. USA 95 (1998) 3949–3953.
[9] J. Björkman, I. Nagaev, O.G. Berg, D. Hughes, D.I. Andersson, Effects of environment on compensatory mutations to ameliorate costs of antibiotic resistance, Science 287 (2000) 1479–1482.
[10] J. Björkman, P. Samuelsson, D.I. Andersson, D. Hughes, Novel ribosomal mutations affecting translational accuracy, antibiotic resistance

and virulence of *Salmonella typhimurium*, Mol. Microbiol. 31 (1999) 53–58.
[11] S.J. Blance, N.L. Williams, Z.A. Preston, J. Bishara, M.S. Smyth, A. Maxwell, Temperature-sensitive suppressor mutations of the *Escherichia coli* DNA gyrase B protein, Protein Sci. 9 (2000) 1035–1037.
[12] G. Bleiber, M. Munoz, A. Ciuffi, P. Meylan, A. Telenti, Individual contributions of mutant protease and reverse transcriptase to viral infectivity, replication, and protein maturation of antiretroviral drug-resistant human immunodeficiency virus type 1, J. Virol. 75 (2001) 3291–3300.
[13] M. Blot, B. Hauer, G. Monnet, The Tn*5* bleomycin resistance gene confers improved survival and growth advantage on *Escherichia coli*, Mol. Gen. Genet. 242 (1994) 595–601.
[14] A.M. Borman, S. Paulous, F. Clavel, Resistance of human immunodeficiency virus type 1 to protease inhibitors: Selection of resistance mutations in the presence and absence of the drug, J. Gen. Virol. 77 (Pt 3) (1996) 419–426.
[15] J.E. Bouma, R.E. Lenski, Evolution of a bacteria/plasmid association, Nature 335 (1988) 351–352.
[16] P.L. Boyer, H.Q. Gao, S.H. Hughes, A mutation at position 190 of human immunodeficiency virus type 1 reverse transcriptase interacts with mutations at positions 74 and 75 via the template primer, Antimicrob. Agents Chemother. 42 (1998) 447–452.
[17] J.J. Bull, M.R. Badgett, H.A. Wichman, Big-benefit mutations in a bacteriophage inhibited with heat, Mol. Biol. Evol. 17 (2000) 942–950.
[18] C.L. Burch, L. Chao, Evolution by small steps and rugged landscapes in the RNA virus phi6, Genetics 151 (1999) 921–927.
[19] D. Butcher, Muller's ratchet, epistasis and mutation effects, Genetics 141 (1995) 431–437.
[20] L. Chao, Fitness of RNA virus decreased by Muller's ratchet, Nature 348 (1990) 454–455.
[21] D.K. Clarke, E.A. Duarte, A. Moya, S.F. Elena, E. Domingo, J. Holland, Genetic bottlenecks and population passages cause profound fitness differences in RNA viruses, J. Virol. 67 (1993) 222–228.
[22] J.H. Condra, W.A. Schleif, O.M. Blahy, L.J. Gabryelski, D.J. Graham, J.C. Quintero, A. Rhodes, H.L. Robbins, E. Roth, M. Shivaprakash, et al., In vivo emergence of HIV-1 variants resistant to multiple protease inhibitors, Nature 374 (1995) 569–571.
[23] F.H.C. Crick, L. Barnett, S. Brenner, R.J. Watts-Tobin, The general nature of the genetic code for proteins, Nature 192 (1961) 1227–1232.
[24] W.D. Crill, H.A. Wichman, J.J. Bull, Evolutionary reversals during viral adaptation to alternating hosts, Genetics 154 (2000) 27–37.
[25] C. Dahlberg, L. Chao, Amelioration of the cost of conjugative plasmid carriage in *Escherichia coli* K12, Genetics 165 (2003) 1641–1649.
[26] A.G. Davies, A.Y. Game, Z. Chen, T.J. Williams, S. Goodall, J.L. Yen, J.A. McKenzie, P. Batterham, Scalloped wings is the *Lucilia cuprina* Notch homologue and a candidate for the modifier of fitness and asymmetry of diazinon resistance, Genetics 143 (1996) 1321–1337.
[27] M. de la Pena, S.F. Elena, A. Moya, Effect of deleterious mutation-accumulation on the fitness of RNA bacteriophage MS2, Evolution 54 (2000) 686–691.
[28] M.T. Dixon, D.M. Hillis, Ribosomal RNA secondary structure: Compensatory mutations and implications for phylogenetic analysis, Mol. Biol. Evol. 10 (1993) 256–267.
[29] E. Duarte, D. Clarke, A. Moya, E. Domingo, J. Holland, Rapid fitness losses in mammalian RNA virus clones due to Muller's ratchet, Proc. Natl. Acad. Sci. USA 89 (1992) 6015–6019.
[30] J.E. Eid, B. Sollner-Webb, Homologous recombination in the tandem calmodulin genes of *Trypanosoma brucei* yields multiple products: Compensation for deleterious deletions by gene amplification, Genes Dev. 5 (1991) 2024–2032.
[31] S.F. Elena, M. Davila, I.S. Novella, J.J. Holland, E. Domingo, A. Moya, Evolutionary dynamics of fitness recovery from the debilitating effects of Muller's ratchet, Evolution 52 (1998) 309–314.
[32] S.F. Elena, F. Gonzalez-Candelas, I.S. Novella, E.A. Duarte, D.K. Clarke, E. Domingo, J.J. Holland, A. Moya, Evolution of fitness in experimental populations of vesicular stomatitis virus, Genetics 142 (1996) 673–679.
[33] C. Escarmis, M. Davila, N. Charpentier, A. Bracho, A. Moya, E. Domingo, Genetic lesions associated with Muller's ratchet in an RNA virus, J. Mol. Biol. 264 (1996) 255–267.
[34] C. Escarmis, M. Davila, E. Domingo, Multiple molecular pathways for fitness recovery of an RNA virus debilitated by operation of Muller's ratchet, J. Mol. Biol. 285 (1999) 495–505.
[35] C. Escarmis, G. Gomez-Mariano, M. Davila, E. Lazaro, E. Domingo, Resistance to extinction of low fitness virus subjected to plaque-to-plaque transfers: Diversification by mutation clustering, J. Mol. Biol. 315 (2002) 647–661.
[36] S. Estes, M. Lynch, Rapid fitness recovery in mutationally degraded lines of *Caenorhabditis elegans*, Evolution 57 (2003) 1022–1030.
[37] J. Felsenstein, The evolutionary advantage of recombination, Genetics 78 (1974) 737–756.
[38] C. Fermer, G. Swedberg, Adaptation to sulfonamide resistance in *Neisseria meningitidis* may have required compensatory changes to retain enzyme function: Kinetic analysis of dihydropteroate synthases from *N. meningitidis* expressed in a knockout mutant of *Escherichia coli*, J. Bacteriol. 179 (1997) 831–837.
[39] J.D. Fry, P.D. Keightley, S.L. Heinsohn, S.V. Nuzhdin, New estimates of the rates and effects of mildly deleterious mutation in *Drosophila melanogaster*, Proc. Natl. Acad. Sci. USA 96 (1999) 574–579.
[40] P. Funchain, A. Yeung, J.L. Stewart, R. Lin, M.M. Slupska, J.H. Miller, The consequences of growth of a mutator strain of *Escherichia coli* as measured by loss of function among multiple gene targets and loss of fitness, Genetics 154 (2000) 959–970.
[41] H. Gatanaga, Y. Suzuki, H. Tsang, K. Yoshimura, M.F. Kavlick, K. Nagashima, R.J. Gorelick, S. Mardy, C. Tang, M.F. Summers et al., Amino acid substitutions in Gag protein at non-cleavage sites are indispensable for the development of a high multitude of HIV-1 resistance against protease inhibitors, J. Biol. Chem. 277 (2002) 5952–5961.
[42] S.H. Gillespie, O.J. Billington, A. Breathnach, T.D. McHugh, Multiple drug-resistant *Mycobacterium tuberculosis*: Evidence for changing fitness following passage through human hosts, Microb. Drug. Resist. 8 (2002) 273–279.
[43] I. Gustafsson, O. Cars, D.I. Andersson, Fitness of antibiotic resistant *Staphylococcus epidermidis* assessed by competition on the skin of human volunteers, J. Antimicrob. Chemother. 52 (2003) 258–263.
[44] J.M. Hancock, D. Tautz, G.A. Dover, Evolution of the secondary structures and compensatory mutations of the ribosomal RNAs of *Drosophila melanogaster*, Mol. Biol. Evol. 5 (1988) 393–414.
[45] D.K. Hanson, D.M. Tiede, S.L. Nance, C.H. Chang, M. Schiffer, Site-specific and compensatory mutations imply unexpected pathways for proton delivery to the QB binding site of the photosynthetic reaction center, Proc. Natl. Acad. Sci. USA 90 (1993) 8929–8933.
[46] P.E. Hartman, J.R. Roth, Mechanisms of suppression, Adv. Genet. 17 (1973) 1–105.
[47] R.B. Helling, T. Kinney, J. Adams, The maintenance of plasmid-containing organisms in populations of *Escherichia coli*, J. Gen. Microbiol. 123 (1981) 129–141.
[48] Y.M. Hou, P. Schimmel, Functional compensation of a recognition-defective transfer RNA by a distal base pair substitution, Biochemistry 31 (1992) 10310–10314.
[49] W. Huang, A. Gamarnik, K. Limoli, C.J. Petropoulos, J.M. Whitcomb, Amino acid substitutions at position 190 of human immunodeficiency virus type 1 reverse transcriptase increase susceptibility to delavirdine and impair virus replication, J. Virol. 77 (2003) 1512–1523.
[50] W. Huang, T. Palzkill, A natural polymorphism in beta-lactamase is a global suppressor, Proc. Natl. Acad. Sci. USA 94 (1997) 8801–8806.
[51] U. Johanson, A. Aevarsson, A. Liljas, D. Hughes, The dynamic structure of EF-G studied by fusidic acid resistance and internal revertants, J. Mol. Biol. 258 (1996) 420–432.

[52] T.T. Kibota, M. Lynch, Estimate of the genomic mutation rate deleterious to overall fitness in *E. coli*, Nature 381 (1996) 694–696.
[53] H.W. Kim, T.J. Shen, D.P. Sun, N.T. Ho, M. Madrid, M.F. Tam, M. Zou, P.F. Cottam, C. Ho, Restoring allosterism with compensatory mutations in hemoglobin, Proc. Natl. Acad. Sci. USA 91 (1994) 11547–11551.
[54] M. Kimura, Some models of neutral evolution, compensatory evolution, and the shifting balance process, Theor. Popul. Biol. 37 (1990) 150–158.
[55] D.A. Kirby, S.V. Muse, W. Stephan, Maintenance of pre-mRNA secondary structure by epistatic selection, Proc. Natl. Acad. Sci. USA 92 (1995) 9047–9051.
[56] J.P. Kleim, M. Rosner, I. Winkler, A. Paessens, R. Kirsch, Y. Hsiou, E. Arnold, G. Riess, Selective pressure of a quinoxaline nonnucleoside inhibitor of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT) on HIV-1 replication results in the emergence of nucleoside RT-inhibitor-specific (RT Leu-74–>Val or Ile and Val-75–>Leu or Ile) HIV-1 mutants, Proc. Natl. Acad. Sci. USA 93 (1996) 34–38.
[57] J. Klovins, N.A. Tsareva, M.H. de Smit, V. Berzins, J. van Duin, Rapid evolution of translational control mechanisms in RNA genomes, J. Mol. Biol. 265 (1997) 372–384.
[58] A.S. Kondrashov, Deleterious mutations and the evolution of sexual reproduction, Nature 336 (1988) 435–440.
[59] C.G. Kurland, D. Hughes, M. Ehrenberg, Limitations of translational accuracy, in: F.C. Neidhardt (Ed.), *Escherichia coli* and *Salmonella*: Cellular and Molecular Biology, second ed., American Society for Microbiology, Washington, DC, 1996, pp. 979–1004.
[60] R. Lande, Risk of population extinction form fixation of new deleterious mutations, Evolution 48 (1994) 1460–1469.
[61] R. Lande, Risk of population extinction from fixation of deleterious and reverse mutations, Genetica 103 (1998) 21–27.
[62] E. Lazaro, C. Escarmis, E. Domingo, S.C. Manrubia, Modeling viral genome fitness evolution associated with serial bottleneck events: evidence of stationary states of fitness, J. Virol. 76 (2002) 8675–8681.
[63] T. Lenormand, T. Guillemaud, D. Bourguet, M. Raymond, Appearance and sweep of a gene duplication: Adaptive response and potential for a new function in the mosquito *Culex pipiens*, Evolution 52 (1998) 1705–1712.
[64] R.E. Lenski, Bacterial evolution and the cost of antibiotic resistance, Intern. Microbiol. 1 (1998) 265–270.
[65] R.E. Lenski, S.C. Simpson, T.T. Nguyen, Genetic analysis of a plasmid-encoded, host genotype-specific enhancement of bacterial fitness, J. Bacteriol. 176 (1994) 3140–3147.
[66] B.R. Levin, The accessory genetic elements of bacteria: Existence conditions and (co)evolution, Curr. Opin. Genet. Dev. 3 (1993) 849–854.
[67] B.R. Levin, V. Perrot, N. Walker, Compensatory mutations, antibiotic resistance and the population genetics of adaptive evolution in bacteria, Genetics 154 (2000) 985–997.
[68] C. Liang, L. Rong, M. Laughrea, L. Kleiman, M.A. Wainberg, Compensatory point mutations in the human immunodeficiency virus type 1 Gag region that are distal from deletion mutations in the dimerization initiation site can restore viral replication, J. Virol. 72 (1998) 6629–6636.
[69] M. Lynch, R. Burger, D. Butcher, W. Gabriel, The mutational meltdown in asexual populations, J. Hered. 84 (1993) 339–344.
[70] M. Lynch, J. Conery, R. Bürger, Mutational meltdown in sexual populations, Evolution 49 (1995) 1067–1080.
[71] M. Lynch, W. Gabriel, Mutation load and the survival of small populations, Evolution 44 (1990) 1725–1737.
[72] M. MacVanin, J. Björkman, S. Eriksson, M. Rhen, D.I. Andersson, D. Hughes, Fusidic acid-resistant mutants of *Salmonella enterica* serovar Typhimurium with low fitness in vivo are defective in RpoS induction, Antimicrob. Agents Chemother. 47 (2003) 3743–3749.
[73] M. MacVanin, U. Johanson, M. Ehrenberg, D. Hughes, Fusidic acid-resistant EF-G perturbs the accumulation of ppGpp, Mol. Microbiol. 37 (2000) 98–107.
[74] S. Maisnier-Patin, O.G. Berg, L. Liljas, D.I. Andersson, Compensatory adaptation to the deleterious effect of antibiotic resistance in *Salmonella typhimurium*, Mol. Microbiol. 46 (2002) 355–366.
[75] F. Mammano, C. Petit, F. Clavel, Resistance-associated loss of viral fitness in human immunodeficiency virus type 1: Phenotypic analysis of protease and gag coevolution in protease inhibitor-treated patients, J. Virol. 72 (1998) 7632–7637.
[76] J.P. Maskell, A.M. Sefton, L.M. Hall, Multiple mutations modulate the function of dihydrofolate reductase in trimethoprim-resistant *Streptococcus pneumoniae*, Antimicrob. Agents Chemother. 45 (2001) 1104–1108.
[77] M.G. Mateu, A.R. Fersht, Mutually compensatory mutations during evolution of the tetramerization domain of tumor suppressor p53 lead to impaired hetero-oligomerization, Proc. Natl. Acad. Sci. USA 96 (1999) 3595–3599.
[78] J.A. McKenzie, P. Batterham, Predicting insecticide resistance: Mutagenesis, selection and response, Philos. Trans. R. Soc. London B Biol. Sci. 353 (1998) 1729–1734.
[79] R.I. Modi, L.H. Castilla, S. Puskas-Rozsa, R.B. Helling, J. Adams, Genetic changes accompanying increased fitness in evolving populations of *Escherichia coli*, Genetics 130 (1992) 241–249.
[80] F.B. Moore, D.E. Rozen, R.E. Lenski, Pervasive compensatory adaptation in *Escherichia coli*, Proc. R. Soc. London B Biol. Sci. 267 (2000) 515–522.
[81] H.J. Muller, The relation of recombination to mutational advance, Mutat. Res. 1 (1964) 2–9.
[82] E.J. Murgola, tRNA, suppression, and the code, Annu. Rev. Genet. 19 (1985) 57–80.
[83] I. Nagaev, J. Björkman, D.I. Andersson, D. Hughes, Biological cost and compensatory evolution in fusidic acid-resistant *Staphylococcus aureus*, Mol. Microbiol. 40 (2001) 433–439.
[84] M.C. Newstein, R.C. Desrosiers, Effects of reverse-transcriptase mutations M184V and E89G on simian immunodeficiency virus in Rhesus monkeys, J. Infect. Dis. 184 (2001) 1262–1267.
[85] M. Nijhuis, R. Schuurman, D. de Jong, J. Erickson, E. Gustchina, J. Albert, P. Schipper, S. Gulnik, C.A. Boucher, Increased fitness of drug resistant HIV-1 protease as a result of acquisition of compensatory mutations during suboptimal therapy, Aids 13 (1999) 2349–2359.
[86] P.V. Nikolova, K.B. Wong, B. DeDecker, J. Henckel, A.R. Fersht, Mechanism of rescue of common p53 cancer mutations by second-site suppressor mutations, EMBO J. 19 (2000) 370–378.
[87] I.S. Novella, C.L. Hershey, C. Escarmis, E. Domingo, J.J. Holland, Lack of evolutionary stasis during alternating replication of an arbovirus in insect and mammalian cells, J. Mol. Biol. 287 (1999) 459–465.
[88] I. Olivares, V. Sanchez-Merino, M.A. Martinez, E. Domingo, C. Lopez-Galindez, L. Menendez-Arias, Second-site reversion of a human immunodeficiency virus type 1 reverse transcriptase mutant that restores enzyme function and replication capacity, J. Virol. 73 (1999) 6293–6298.
[89] R.C. Olsthoorn, N. Licis, J. van Duin, Leeway and constraints in the forced evolution of a regulatory RNA helix, EMBO J. 13 (1994) 2660–2668.
[90] R.C. Olsthoorn, J. van Duin, Evolutionary reconstruction of a hairpin deleted from the genome of an RNA virus, Proc. Natl. Acad. Sci. USA 93 (1996) 12256–12261.
[91] M.C. Orencia, J.S. Yoon, J.E. Ness, W.P. Stemmer, R.C. Stevens, Predicting the emergence of antibiotic resistance by directed evolution and structural analysis, Nat. Struct. Biol. 8 (2001) 238–242.
[92] J. Parsch, S. Tanda, W. Stephan, Site-directed mutations reveal long-range compensatory interactions in the *Adh* gene of *Drosophila melanogaster*, Proc. Natl. Acad. Sci. USA 94 (1997) 928–933.
[93] A. Poon, S.P. Otto, Compensating for our load of mutations: Freezing the meltdown of small populations, Evolution 54 (2000) 1467–1479.
[94] G. Pozzi, M. Meloni, E. Iona, G. Orru, O.F. Thoresen, M.L. Ricci, M.R. Oggioni, L. Fattorini, G. Orefici, *rpoB* mutations in multidrug-

resistant strains of *Mycobacterium tuberculosis* isolated in Italy, J. Clin. Microbiol. 37 (1999) 1197–1199.
[95] M. Raymond, C. Berticat, M. Weill, N. Pasteur, C. Chevillon, Insecticide resistance in the mosquito *Culex pipiens*: What have we learned about adaptation?, Genetica 112-113 (2001) 287–296.
[96] M.G. Reynolds, Compensatory evolution in rifampin-resistant *Escherichia coli*, Genetics 156 (2000) 1471–1481.
[97] D. Rokyta, M.R. Badgett, I.J. Molineux, J.J. Bull, Experimental genomic evolution: Extensive compensation for loss of DNA ligase activity in a virus, Mol. Biol. Evol. 19 (2002) 230–238.
[98] D. Romero, R. Palacios, Gene amplification and genomic plasticity in prokaryotes, Annu. Rev. Genet. 31 (1997) 91–111.
[99] F. Rousset, M. Pelandakis, M. Solignac, Evolution of compensatory substitutions through G. U intermediate state in Drosophila rRNA, Proc. Natl. Acad. Sci. USA 88 (1991) 10032–10036.
[100] T. Ruusala, D. Andersson, M. Ehrenberg, C.G. Kurland, Hyper-accurate ribosomes inhibit growth, EMBO J. 3 (1984) 2575–2580.
[101] S.G. Sarafianos, K. Das, C. Tantillo, A.D. Clark, J. Ding, J.M. Whitcomb, P.L. Boyer, S.H. Hughes, E. Arnold, Crystal structure of HIV-1 reverse transcriptase in complex with a polypurine tract RNA:DNA, EMBO J. 20 (2001) 1449–1461.
[102] S.J. Schrag, V. Perrot, B.R. Levin, Adaptation to the fitness costs of antibiotic resistance in *Escherichia coli*, Proc. R. Soc. London B Biol. Sci. 264 (1997) 1287–1291.
[103] D.R. Sherman, K. Mdluli, M.J. Hickey, T.M. Arain, S.L. Morris, C.E. Barry 3rd, C.K. Stover, Compensatory *ahpC* gene expression in isoniazid-resistant *Mycobacterium tuberculosis*, Science 272 (1996) 1641–1643.
[104] V. Sideraki, W. Huang, T. Palzkill, H.F. Gilbert, A secondary drug resistance mutation of TEM-1 beta-lactamase that suppresses misfolding and aggregation, Proc. Natl. Acad. Sci. USA 98 (2001) 283–288.
[105] W. Stephan, D.A. Kirby, RNA folding in Drosophila shows a distance effect for compensatory fitness interactions, Genetics 135 (1993) 97–103.
[106] B. Taddeo, F. Carlini, P. Verani, A. Engelman, Reversion of a human immunodeficiency virus type 1 integrase mutant at a second site restores enzyme function and virus infectivity, J. Virol. 70 (1996) 8277–8284.
[107] H. Teotonio, M.R. Rose, Perspective: Reverse evolution, Evol. Int. J. Org. Evol. 55 (2001) 653–660.
[108] L.L. Vassilieva, A.M. Hook, M. Lynch, The fitness effects of spontaneous mutations in *Caenorhabditis elegans*, Evolution 54 (2000) 1234–1246.
[109] G.P. Wagner, W. Gabriel, Quantitative variation in finite parthenogenetic populations: what stops Muller's ratchet in the absence of recombination?, Evolution 44 (1990) 715–731.
[110] X. Wang, G. Minasov, B.K. Shoichet, Evolution of an antibiotic resistance enzyme constrained by stability and activity trade-offs, J. Mol. Biol. 320 (2002) 85–95.
[111] A.M. Whatmore, N. Cook, G.A. Hall, S. Sharpe, E.W. Rud, M.P. Cranage, Repair and evolution of nef in vivo modulates simian immunodeficiency virus virulence, J. Virol. 69 (1995) 5117–5123.
[112] M.C. Whitlock, Fixation of new alleles and the extinction of small populations: Drift load, beneficial alleles, and sexual selection, Evolution 54 (2000) 1855–1861.
[113] M.C. Whitlock, S.P. Otto, The panda and the phage: Compensatory mutations and the persistence of small populations, Trends Ecol. Evol. 14 (1999) 295–296.
[114] C.O. Wilke, R.E. Lenski, C. Adami, Compensatory mutations cause excess of antagonistic epistasis in RNA secondary structure folding, BMC Evol. Biol. 3 (2003) 3.
[115] J.W. Wray, W.A. Baase, J.D. Lindstrom, L.H. Weaver, A.R. Poteete, B.W. Matthews, Structural analysis of a non-contiguous second-site revertant in T4 lysozyme shows that increasing the rigidity of a protein can enhance its stability, J. Mol. Biol. 292 (1999) 1111–1120.
[116] S. Wright, Stochastic processes in evolution, in: J. Garland (Ed.), Stochastic Models in Medicine and Biology, Univ. of Wisconsin Press, Madison, WI, 1964.
[117] S. Wright, The shifting balance theory and macroevolution, Annu. Rev. Genet. 16 (1982) 1–19.
[118] E. Yuste, S. Sanchez-Palomino, C. Casado, E. Domingo, C. Lopez-Galindez, Drastic fitness loss in human immunodeficiency virus type 1 upon serial bottleneck events, J. Virol. 73 (1999) 2745–2751.
[119] C. Zeyl, M. Mizesko, J.A. de Visser, Mutational meltdown in laboratory yeast populations, Evolution 55 (2001) 909–917.
[120] Y.M. Zhang, H. Imamichi, T. Imamichi, H.C. Lane, J. Falloon, M.B. Vasudevachari, N.P. Salzman, Drug resistance during indinavir therapy is caused by mutations in the protease gene and in its Gag substrate cleavage sites, J. Virol. 71 (1997) 6662–6670.
[121] E. Zuckerkandl, Neutral and nonneutral mutations: The creative mix–evolution of complexity in gene interaction systems, J. Mol. Evol. 44 (Suppl. 1) (1997) S2–8.
[122] E. Zuckerkandl, Intrinsically driven changes in gene interaction complexity: I. Growth of regulatory complexes and increase in number of genes, J. Mol. Evol. 53 (2001) 539–554.