EVOLUTION OF BACTERIOPHAGE Qβ UNDER THE SELECTIVE PRESSURE OF INCREASED ERROR RATE. E. Lázaro$^1$ and M. Arribas$^2$, $^1$(Centro de Astrobiología, Ctra de Ajalvir Km. 4, 28850 Torrejón de Ardoz, Madrid, Spain,izarolm@inta.es), $^2$(Centro de Astrobiología, Ctra de Ajalvir Km. 4, 28850 Torrejón de Ardoz, Madrid, Spain).

Introduction: The error rate at which a population replicates has important implications on its ability to adapt to the changes that continuously occur in the environment. Although most mutations are deleterious, natural selection increases the representation of those having beneficial effects, leading to fitness increases in the particular environment in which replication takes place.

RNA viruses replicate their genomes with very high error rates, in the order of $10^{-3}$ to $10^{-5}$ errors per nucleotide and round of copy. As a consequence, they give rise to populations constituted by an extremely heterogeneous mutant spectrum with an elevated capacity of adaptation to almost any change in the selective pressures [1]. These populations are termed virus quasispecies by analogy to the molecular quasispecies defined in the context of the origin of life to describe the stationary state attained by replicator populations at mutation-selection equilibrium [2]. Therefore, RNA viruses constitute an excellent model to identify universal principles governing the molecular basis of adaptation in both cellular organisms and primitive replicators.

Replication at high error rates also has negative consequences, and quasispecies theory predicts the existence of an error threshold that corresponds to the value of the error rate above which the mutational force cannot be counteracted for the action of selection. Crossing the error threshold supposes the transition to a random distribution of mutants through a process known as error catastrophe [3]. A point that deserves great interest is that replication at higher than standard mutation rates constitutes a selective pressure by itself, and RNA viruses have developed strategies to counteract its negative effects. These adaptation mechanisms involve that selective processes are still effective for a range of error rate values above those used habitually by the virus.

Objectives: In this work we have made a detailed study of the genetic and phenotypic characteristics of the populations generated by an RNA virus replicating in presence of different concentrations of a mutagen that increases the error rate. We have chosen the bacteriophage Qβ as experimental model because it has a very small size genome (4217 nucleotides) encoding only 4 proteins, and with many cis-acting elements that are required to complete the full replication cycle of the phage. The cis-acting RNA elements overlap extensively with the protein coding domains, causing that the genome is highly compacted and presenting many constraints to the accumulation of mutations. All this converts to bacteriophage Qβ in an adequate system to study the action of selection under conditions of increased mutagenesis. We have used the mutagen 5-azacytidine (AZC), which is able to produce the extinction of the phage when it is propagated using small population sizes and high mutagen concentration [4].

Results: Bacteriophage Qβ can be maintained replicating in the presence of moderate concentrations of AZC when it is transmitted using large population sizes. The distribution of mutations in the viable genomes composing the phage populations is non-homogeneous, suggesting that survival takes place through an increase of the purifying selection that is able to eliminate the lowest fitness genomes. Additional passages of the virus in the presence of increased AZC concentrations lead to the generation of populations with reduced sensitivity to the action of the mutagen, which is associated to a reduction in the mutation frequency. The determination of the consensus sequence in the resistant populations shows that substitution A1746T (located in one of the structural proteins) appears at early passages and is the only that becomes fixed. At later passages, several additional mutations, which are located in the replicase gene, appear. In spite that these mutations have selective value in the presence of mutagen, they do not become fixed in the consensus sequence, remaining at variable frequencies in the population. Our results suggest that the fixation of beneficial mutations arising in highly mutagenized populations is hindered due to the possible deleterious effects of other accompanying mutations. The competition among genomes carrying different beneficial mutations complicates the situation even more, leading to the generation of populations with a high degree of complexity in which each individual genome has a different level of tolerance to AZC.