SUMMARY

Life expectancy in the 20th century has been greatly increased in the Western society but it has been paralleled by a progressive increase in the incidence of age-related diseases, including neurodegeneration and dementia, which interfere with the quality of life. Understanding mechanisms underlying longevity, and endeavor towards the specific goals of alleviating frailty in old age, is thus becoming a hot issue.

The present thesis was aimed at investigating the mechanisms underlying the extended longevity by characterizing the p66Shc-/- mouse. P66Shc is a gene encoding for an inducible redox enzyme which, when activated by stress, produces ROS (H_2O_2) to trigger apoptosis. The deletion of it leads therefore to a condition of reduced oxidative stress and is able to increase by 30% the lifespan in mice (Giorgio et al., 2007). In addition, a role for this gene in regulating the effect of insulin on energy metabolism (in mice) has been recently described. In line with such a role, the p66Shc gene is found expressed in the pituitary and in the brain with levels being high early during development, when cells grow and differentiate. Aberrant hypothalamic-pituitary-adrenal (HPA) axis activity and elevated glucocorticoid (GC) levels have been associated to aging and age-related neurodegenerative pathologies. Evidence is mounting that stress and oxidative stress might act synergistically to induce or exacerbate the neuronal decay associated with aging. A growing body of evidence has shown a redox regulation of the function of the glucocorticoid receptors which operate as nuclear transcription factors. Thus, the main question addressed in this research was whether the complex interactions linking oxidative stress and the neuroendocrine system might represent a major determinant for the aging process in p66Shc-/- mice. Specifically, the reduced levels of reactive oxygen species, characteristic of these long-lived subjects, were expected to result in a more efficient regulation of the hypothalamic-pituitary-adrenal axis and hence in a delay of the aging process. To this aim the contribution of p66Shc to behavioral and neuroendocrine regulations was tested from early post-natal life to senescence.

In Chapter 2 the effect is described of the lack of the p66Shc gene on maternal behavior and on the overall fitness. Maternal behavior during early post-natal life is likely to contribute in shaping the behavioral/emotional (Chapters 4 and 5) and the neuroendocrine phenotype (Chapter 3) of the p66Shc-/- mice via a mechanism mediated by maternal metabolic demand. From postnatal day 1 to 8 p66Shc-/- pups experienced abrupt changes in maternal care when compared to controls. In particular, maternal behavior of mutant dams was constrained and redirected as a function of the feeding pattern driven by the disruption of the p66Shc-mediated metabolic pathway. This pattern may be assimilated to the previously described experimental handling condition or to an ecological scenario in which food is not available ad libitum. P66Shc-/- mice have been recently found to be characterized by high metabolic rate and low fat accumulation, thus it is conceivable to hypothesize that changes in maternal care are related to a higher metabolic need, especially during a demanding challenge such as
Notably, mutant dams showed a higher rate of cannibalism compared to wild type. This behavior could be a consequence of the peculiar metabolic needs of this genotype. In addition, p66shc mutants were characterized by a reduced fitness over repeated breeding cycles and under stressful conditions, together with an earlier onset of puberty.

These data suggest that a reason for the p66shc gene for being conserved might rely on its role on reproduction and point to p66shc as a candidate genetic determinant in the trade-off between fertility and lifespan.

In Chapter 3 studies are described aimed at investigating the neuroendocrine function of KO mice, possibly as a key factor underlying both the emotional phenotype as well as the longevity of mutant subjects. We found that the neuroendocrine function of the mutants (at adulthood) did not differ between the two genotypes as expected, as shown by corticosterone (CORT) response to restraint stress and to LPS challenge. However, a mild hyperdrive of the HPA axis was found following a dexamethasone suppression test, which could reflect a fine tuning of neuroendocrine function and can be related to the overall healthier and long-lived phenotype of the mutants. For the first time markers of oxidative stress were measured in the CNS of these mutants. When changes in hippocampal oxidative status were taken into account following LPS, KO subjects did not present increased levels of isoprostanes (15-F₂IsopP). At the same time, the neurotrophin brain-derived-neurotrophic factor (BDNF) was selectively increased in WT subjects, while levels of prostaglandins (PGE₂) were reduced only in the mutants, possibly as a result of reduced neuronal excitation in response to stress in the latter group.

It was concluded that the greater resilience to stress-induced changes in the p66shc-/- mutants might underlie the better health status and the greater longevity characterizing these mice (Chapters 4 and 5).

In Chapters 4 and 5 we assessed the effects of the lack of the p66shc gene on the behavioral phenotype of mutant subjects at different ages, from adulthood to senescence focusing on cognitive, emotional and nociceptive traits as landmarks of aging. Mutant mice were characterized by a smoother age-dependent change in the behavioral profile, lower emotionality and better cognitive abilities at adulthood, together with a general healthier phenotype in aged individuals. Interestingly, these behavioral traits in KO adult subjects were accompanied by increased basal levels of BDNF in the hippocampus, as well as decreased levels of oxidative stress markers in the same brain area.

Data on the behavioral phenotype point to p66shc as another target gene to study the basic mechanisms involved in neuronal plasticity and emotional disorders, in addition to healthier aging.

Data obtained in the present thesis indicate that in this mouse model, the role of the p66shc gene in determining lifespan is not merely related to its effects on oxidative stress but also to its interaction with metabolic and behavioral signaling cascades which appear as important determinants of healthspan during aging.