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FORM 101
Application for a Grant
PART I

Date: 2001/10/30

Family name of applicant	Given name	Initial(s) of all given names	Personal identification no. (PIN)
Seroude	Laurent	L	45545

Language of application	Time (in hours per month) to be devoted to the proposed research
[X] English [ ] French	120

Type of grant applied for	For Strategic Projects, indicate the Target Area and Sub-area(s), if applicable
Research Grants - Individual	

Title of proposal
Dissection of age-dependent gene expression

Write a maximum of ten (10) key words that describe this proposal. Use commas to separate them.
Drosophila melanogaster, Aging, Regulation of gene expression, Molecular Genetics

Research subject code(s)		Area of application code(s)	
Primary	Secondary	Primary	Secondary
5002	5303	1201	1211

CERTIFICATION REQUIREMENTS

If this proposal involves any of the following, check the box(es) and submit the protocol to the university certification committee.

Research involving humans [ ] Research involving animals [ ] Research involving biohazards [X]

Does any phase of the research described in this proposal a) take place outside an office or laboratory, or b) involve an undertaking as described in Part 1 of Appendix B?

[X] NO [ ] If YES to either question a) or b) – Appendices A and B must be completed

TOTAL AMOUNT REQUESTED FROM NSERC

Year 1	Year 2	Year 3	Year 4	Year 5
79,720	79,720	79,720	79,720	0

SIGNATURES (Refer to instructions "What do signatures mean?")

It is agreed that the general conditions governing grants as outlined in the NSERC *Program Guide for Professors* apply to any grant made pursuant to this application and are hereby accepted by the applicant and the applicant's employing institution.

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The information collected on this form and appendices will be stored in the Personal Information Bank for the appropriate program.

Version française disponible

PROTECTED WHEN COMPLETED

Not Verified

Personal identification no. (PIN)	Family name of applicant
45545	Seroude

SUMMARY OF PROPOSAL FOR PUBLIC RELEASE (Use plain language.)

This plain language summary will be available to the public if your proposal is funded. Although it is not mandatory, you may choose to include your business telephone number and/or your e-mail address to facilitate contact with the public and the media about your research.

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Aging is a biological reality that is familiar to everyone. The fruit fly, Drosophila melanogaster, is an excellent model organism, easy and inexpensive to culture, which has contributed to the understanding of many universal biological processes. For instance, many publications in prestigious journals have reported Drosophila to be a suitable model to study human diseases such as Parkinson's, Alzheimer's, Huntington's, and Adrenoleukodystrophy, which hasten the identification of the cellular processes and molecular pathways affected by these pathologies. Using this model, Dr. Seroude aims to establish a molecular genetics aging research program for addressing key questions such as the relationship between genetic expression and senescence. This research is critical to develop the fundamental research for exploring the biology of aging. The significant growth of the elderly population has important implications for individuals and families as well as for public policy makers. If interventions can be developed that enable the elderly to stay healthy and independent longer, these medical costs would be reduced. The development of such interventions cannot be done rigorously and without undesirable side effects if we do not understand the basic mechanisms of aging. This research program will constitute an excellent opportunity to attract highly qualified researchers, maintain internationally competitive research programs at Queen's University and will bring to Canada a strong and innovative international reputation in this area of research.

RESEARCH ACTIVITY SCHEDULE

(Refer to instructions to see if this section applies to your application. Use additional page(s) if necessary.)

Milestone	Description of activities	Anticipated starting date	Anticipated completion date

Personal identification no. (PIN)	Family name of applicant
45545	Seroude

Before completing this section, **read the instructions** and consult the *Financial Administration* section in the NSERC *Program Guide for Professors* concerning the eligibility of expenditures for the direct costs of research and the regulations governing the use of grant funds. On separate page(s), supply a detailed explanation, and justification, for your proposed expenditures. **Also explain the relationship or difference between this application and all other research support (held or applied for),** and describe any contributions from other sources (if applicable).

PROPOSED EXPENDITURES FOR DIRECT COSTS OF RESEARCH (Include cash expenditures only)

	Year 1	Year 2	Year 3	Year 4	Year 5
1) Salaries and benefits					
a) Students	15,012	15,012	15,012	15,012	0
b) Postdoctoral fellows	0	0	0	0	0
c) Technical/professional assistants	28,708	28,708	28,708	28,708	0
d)	0	0	0	0	0
2) Equipment or facility					
a) Purchase or rental	5,000	5,000	5,000	5,000	0
b) Operation and maintenance costs	0	0	0	0	0
c) User fees	0	0	0	0	0
3) Materials and supplies	29,000	29,000	29,000	29,000	0
4) Travel					
a) Conferences	2,000	2,000	2,000	2,000	0
b) Field work	0	0	0	0	0
c) Collaboration/consultation	0	0	0	0	0
5) Dissemination costs					
a) Publication costs	0	0	0	0	0
b)	0	0	0	0	0
6) Other (specify)					
a)	0	0	0	0	0
b)	0	0	0	0	0
TOTAL PROPOSED EXPENDITURES FOR DIRECT COSTS OF RESEARCH	79,720	79,720	79,720	79,720	0
Total cash contribution from industry (if applicable)					
Total cash contribution from university (if applicable)					
Total cash contribution from other sources (if applicable)	0	0	0	0	0
TOTAL AMOUNT REQUESTED FROM NSERC (transfer to page 1)	79,720	79,720	79,720	79,720	0

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FORM 101, Part II **Research Proposal**

Dissection of age-dependent gene expression

Background and Objectives

Aging is a fundamental biological process that can be defined, measured, described and manipulated. It is a genetically determined, environmentally modulated, event-dependent process and is not a random decay of the body's parts and functions (1-3). However, the complex details of aging mechanisms remain unclear (4, 5). **My long-term goal is to understand the genetic component with a special emphasis on the relationship between genes and aging**. The regulation of the developmental process that allows a single cell to become a complex organism capable of procreation is under tight genetic control. It is well established that a very precise regulation of the expression and combinatorial action of many genes can differentiate a human from a chimpanzee, which has less than 5% differences at the DNA level. Would it be possible that subtle changes in gene expression could result in similarly drastic changes in aging? Although aging may be characterized by the loss of some kinds of homeostasis, it is not associated with a pervasive decline of regulation of gene expression. Gene expression appears to be well regulated even at older ages when biological performance is generally diminished (6, 7). In contrast to the evolutionary ideas that aging is the detritus of the absence of selection after the reproductive period and just represents the organism falling apart, studies of gene expression have shown that aging is associated with the same highly dynamic regulated changes observed during development (7-14). Single gene mutants with extended viability have been found in the nematode *Caenorhabditis elegans* (15-24), the fruit fly *Drosophila melanogaster* (25-28), the yeast *Saccharomyces cerevisiae* (29-31) and the mouse *Mus musculus* (32). The molecular analysis of several of these mutants identified genetic pathways implicated in the regulation of the life span that are strongly suspected to mediate their effect through the regulation of gene expression. Finally, experimental alterations of the life spans of flies and rodents cause corresponding changes in gene activity, establishing a definitive link between progression of age and genetic expression (8, 10, 11, 13, 33).

These observations raise the question of whether gene expression changes are a cause or a consequence of the aging process. The characterization of changes in gene expression cannot distinguish between these two alternatives. **My lab aims to address this question by manipulating age-dependent gene expression and examining the consequences on the aging process**. This is accomplished using the power of Drosophila's genetics, a proven efficient model for elucidating the role of genes and gene expression in many biological processes such as development, immunity, behavior and pathological disorders (34-47).

Novelty, Anticipated Impact and Potential Benefits

My research program constitutes the first full commitment in the aging field to the characterization of the regulatory components responsible for the age-dependent regulation of transcription. The combination of molecular, genetic, and biochemical approaches will provide a large-scale survey of the gene regulation pathways associated with the progression of an organism through aging. My work will establish the information and the molecular genetic tools essential to manipulate gene expression during aging. Finally, it will lead to the identification of genes regulating the rate of aging. Drosophila genes have led to the identification of mammalian cognates, and to an extent no one predicted, many of these cognates have closely related functions in mammals (48). Although the genes identified in this program may not be

sufficient to stop the aging process, they will constitute excellent candidate targets to slow down aging and extend human youth.

Specific Aims

The goals of this proposal are to use the Drosophila model to identify:

1) DNA regulatory sequences responsible for age-dependent gene regulation
2) The genes regulated by these sequences
3) The factors required for their regulatory properties.

These projects are interconnected and the advance of one will facilitate the progress of the others. However, each project can be started independently and does not rely on the others to be successful.

Proposed Research

Identification of DNA regulatory sequences responsible for age-dependent gene regulation

Rationale: Recently developed micro-array technology can be used to examine quantitative changes in gene expression at a genomic level allowing the monitoring of thousands of genes (9-12). Such approach is powerful and fast but it carries limitations and needs to be complemented by genetic methods. To be applicable to the analysis of spatial changes, they require dissecting every tissue and repeating the experiment on the RNA population from the isolated tissue. Though RNA amplification techniques are available, characterizing the gene expression profile of single cell would require enormous resources. Second, gene expression results from the combinatorial action of several regulatory components whose individual activities are not accessible by the micro-array technology. The enhancer-trap technique is a powerful method to identify DNA regulatory sequences *in vivo* (49-51). This genetic technique allows the experimentalist to monitor the transcriptional activity of many regions of the genome (49). This kind of analysis can be used to examine spatial and temporal changes in gene expression at the single cell level and down to the regulatory elements that dictate these changes. This technique is easy and cost effective to dissect at relatively large scale the transcriptional regulatory pathways associated with the aging process.

Experimental design: As a post doctoral fellow, I used the UAS/GAL4 system (52) to established a collection of 500 GAL4 enhancer-trap lines (8). By crossing every line with a line carrying a UAS-lacZ construct encoding the bacterial enzyme ß-galactosidase, the amount and the localization of the activity of the DNA regulatory sequences reported by the GAL4 enhancer trap can be determined across the fly life span by biochemical assay or *in situ* detection. Almost half of the collection has already been analyzed and so far, over 80% of the GAL4 lines exhibit age-related ß-galalactosidase changes. Remarkably the alteration of longevity by mutations or environment does not affect the pattern of change, but only the rate at which they occur. This observation demonstrates that these changes are regulated by physiological age rather than chronological age. Three lines identify regulatory elements with a particularly interesting spatial and temporal pattern of activity. The line *middle age crisis* (*mac*) does not show any expression during development and an extremely low level of lacZ expression restricted to a subset of adult sensory cells until mid-age (25-30 days at 25˚C). After this time the expression is induced in the abdominal oenocytes and keeps increasing until the death of the individual. The line *jumpy* (*jmp*) is restricted to muscle tissues increasing up to 20 days then slowly decreasing while the line DJ634 is limited to fat tissues and a single pair of thoracic muscles with a peak of expression at old age. The analysis of the remaining lines will be pursued but the identification of the DNA regulatory sequences in *mac*, *jmp* and DJ634 will be started. Genomic DNAs have already been isolated and the localization of the insertions of the GAL4 enhancer-trap construct has been determined by the plasmid-rescue method (53). Taking advantage of the recent completion of the *Drosophila* genome (54), the DNA sequence analysis of the genomic region

surrounding the insertion revealed the presence of repeated motifs that may correspond to the regulatory sequences controlling the expression of the GAL4 enhancer-trap. For instance, such motif is found five times around the *mac* insertion and two or three times in three additional genomic locations. PCR primers will be designed to amplify fragments of genomic DNA with or without these conserved motifs. Each fragment will be cloned in a GAL4 vector and transgenic lines will be established and tested for GAL4 expression as described above.

Expected outcome: The enhancer-trap technique has proven its effectiveness in studying developmental processes (55-58) and it can be applied to the genetic dissection of aging (8, 13, 14). The proposed transgenic approach has been extensively and successfully used to identify regulatory sequences important during development (59-61). A candidate gene approach has shown an age-dependent increase of the chaperone proteins HSP70 and HSP22 (33, 62) and a transgenic approach demonstrated that the heat shock response element is necessary for this effect (62, 63). These reports are encouraging and predict that my proposal will indeed lead to the identification of many age-dependent DNA regulatory sequences. In addition, all the GAL4 enhancer-trap lines characterized and the newly generated transgenic lines will provide a very useful collection of age-dependent and tissue-specific GAL4 expression tools. They will be used in the future to manipulate the expression of genes of interest and examine the consequences on the aging process. Since GAL4 expression changes are linked to the physiological age, this collection can also be used as biomarker of aging to monitor the progression of the aging process without having to wait for the death of the individual. Many genetic interventions are known to affect life span, implying that further studies on aging mechanisms hinge upon dissecting genetic mutations that affect aging. Biomarkers are especially suitable for testing such mutations. The mutant should demonstrate a loss or gain of a biomarker at a later or earlier age, as compared with the wild type. Mutants that do so are likely to exhibit a genetic alteration of the aging process.

Potential problems: The experimental procedures used are simple and will not generate any technical challenges. The pattern of regulation observed in the GAL4 enhancer-trap lines might be the result of the combinatorial action of several DNA regulatory elements. To address this issue, the transgenic studies will not be restricted to the sole repeated motifs and larger genomic regions will also be included in the analysis.

Identification of genes under the control of age-dependent DNA regulatory sequences

Rationale: The enhancer-trap methodology has been very effective for detecting age-dependent DNA regulatory elements. The existence of such elements raises the question of which genes are under their control and the biological reason for such regulation. Finding these genes and analyzing their function during aging is the next logical step toward the identification and dissection of age-dependent genetic pathways.

Experimental design: The examination of the genomic region containing the GAL4 enhancer-trap has been done using the data obtained by the Berkeley Drosophila Genome Project. The *jumpy* insertion is in the first intron of a gene encoding a putative tyrosine phosphatase. The *mac* insertion is in a 1kb region separating two genes both encoding a peptidoglycan recognition protein (PGRP). PGRPs are evolutionarily conserved and important for the recognition of bacterial infection and the subsequent activation of the immune response (64-66). It has been recently published that the RNA expression of these two genes is induced specifically by bacterial infection in the adult fly (67). Interestingly, this induction is not observed if the infection is done in the larvae (65), suggesting that these genes are adult-specific. In agreement with this observation we found that the *mac* insertion does not express GAL4 before the adult stage. Finally, the DJ634 insertion is localized close to a gene related to DNA helicases. Unfortunately, none of these genes are present in the micro-array analysis of Drosophila aging (9). Using Northern-blot and *in situ*

hybridization techniques, the temporal and spatial expression of these genes will be determined across the life span.

Expected outcome: Finding genes by screening for their pattern of expression is an efficient method to address biological problems (68-72). This approach will identify age-regulated genes potentially important for the aging process. The closest genes to the GAL4 enhancer-trap insertion in two of the three lines examined so far promise to be biologically relevant to aging.

It is well known that aging is associated with a decline of immune function (4, 73, 74) and recent studies in mice and primates have shown an increase in the RNA expression of genes involved in the inflammatory response (11, 12). The adult specific response to bacterial infection of the PGRP genes surrounding the *middle age crisis* (*mac*) insertion and the absence of GAL4 activity prior to the adult stage suggest that the PGRP genes are indeed subject to age-dependent regulation. The *mac* line can then be used to investigate genetically the relationship between immunity related genetic pathways, the decline of the immune function and the aging process. It will be very easy to mutate the PGRP genes by imprecise excision of the GAL4 enhancer-trap construct. This can also be done cleanly by using the RNAi interference technique (75, 76) in which an UAS-RNAi construct can be controlled by the *mac* GAL4 activity. Therefore I can remove PGRP function specifically in the right tissues at the right time avoiding unspecific effects due to ectopic expression (for instance by interfering with other members of the conserved PGRP family). Taking advantage of the age-dependent GAL expression tools generated in the first project described in this proposal, it will also be possible to manipulate the expression of the PGRP genes by expressing them earlier or later. Two hypotheses can explain the age-dependent increase in PRGP genes expression. Like human skin, during aging fly cuticle may be a less efficient barrier to microbial aggression. Alternatively, it is well know that aging is associated with the accumulation of irreversibly altered molecules such as glycation products (5, 77). When a critical level is reached, such products may induce the immune response. This would be very similar to an auto-immunity defect. This can be easily tested; culturing flies in sterile conditions would not prevent the induction and reciprocally injecting glycation products would induce it in young flies. Both hypotheses would result in the overwhelming of the immune system that would prevent its normal function, make it decline and be an important contribution to the aging process and elderly pathologies.

Aging is also associated with a decline of locomotion and muscle performance (78). Individuals homozygous for the *jumpy* (*jmp*) insertion exhibit an age-dependent locomotion disorder and are short-lived. Interestingly, the *jmp* tyrosine phosphatase active site is closely related to the MTM family defined by the MTM1 protein which has been shown to be mutated in humans affected by myotubular myopathy, a muscle disorder characterized by severe hypotonia and generalized muscle weakness (79). Since the *jmp* GAL4 expression is strictly restricted to muscle tissue, it is probable that the jmp tyrosine phosphatase gene is age-regulated. Inactivating or manipulating the expression of this gene will shed light on its aging-related significance.

Potential problems: Although being time consuming, this project is technically easy and straightforward. Since the same DNA regulatory sequences can act on several genes and at very large map distances, the analysis will also be applied to all the genes within 50kb on either side of the GAL4 enhancer-trap insertion.

Identification of the factors required for the activity of age-dependent DNA regulatory sequences

Rationale: Many genes have significant age-specific changes in their RNA levels with increasing age (80). This proposal will identify such genes and the DNA regulatory sequences controlling these changes. The next logical step will be to characterize the factors acting on these sequences to confer their regulatory properties. Such factors are likely to reveal the molecular basis of the changes associated with the progression of age.

Experimental design: The factors required for the activity of the identified DNA regulatory sequences will be determined by a combination of genetic and biochemical screens. The genetic screen, using over-expression (81), will aim to find genes that prevent or modify age-dependent enhancer activity. Simple crosses will be done to construct “indicator” fly strains homozygous for a UAS-GFP construct and the *mac*, *jmp* or DJ634 GAL4 enhancer-traps. These strains will allow a quick and easy scoring of GFP fluorescence on the same individuals across their life span thereby decreasing the number of flies maintained during the screening. A collection of EP strains will be established, each strain carrying a random insertion of a P-element vector (EP element) carrying a GAL4-regulated promoter oriented to transcribe flanking genomic sequences. The progeny of crosses between EP and the indicator strains will then be examined for GFP expression across life span. EP element insertions preventing or modifying GFP expression will be selected and analyzed to identify the over-expressed gene affected by the EP insertion. The biochemical approach will involve screening age-specific Drosophila cDNA expression libraries for molecules that bind to the DNA regulatory sequences identified in the first project described in this proposal. The corresponding genes will be examined *in vivo* by inactivation or over-expression to find which ones are responsible for age-dependent regulation.

Expected outcome: This over-expression protocol has recently been reported to be an efficient method to find genes important for development or the prevention of neurodegeneration (44, 82, 83). This approach will identify genes that modify the regulatory properties of the age-dependent DNA regulatory sequences. In combination with the biochemical approach, this method should identify the factors that are acting directly by binding to these sequences. In addition, the genes identified that delay or accelerate the pattern of activity will be candidate “true aging” genes since they do not alter the nature of an age-dependent change but specifically affect the rate at which this change occurs.

Potential problems: The biochemical approach relies on the completion of the first project, however it will be possible to establish and use the technique with the genomic DNA regions surrounding the GAL4 enhancer-trap insertions. Using larger regions will probably increase the occurrence of false-positive clones but this problem can be minimized by the simultaneous use of the genetic screen approach. Genes that will be selected by these independent genetic and biochemical screens will be given the highest priority for future investigations.

Training of highly qualified personnel

I have had over 10 years of experience in all aspects of Drosophila molecular genetics and I am very familiar with the techniques involved in this proposal. Drosophila has recently become of great interest for investigating the basic mechanisms underlying human diseases (84, 85). In the instance of the Adrenoleukodystrophy disease, it has been shown that therapeutic treatment used in humans is effective to cure the fly model (40). This kind of observation has prompted pharmaceutical companies (Brystol-Myers Applied Genomics, Exelixis, Develogen, Cambria Bioscience) to develop the fly model as a rapid and inexpensive procedure to screen for drugs of therapeutic interest for humans (Alzheimer disease, obesity) or economically important species (insecticides, pesticides). Such companies are currently seeking fly molecular geneticists as indicated by their increased presence in the major Drosophila-related research conferences. The students and research assistant involved in this research will acquire a Drosophila-related knowledge that will make them attractive to these companies. My research program uses technologies (DNA cloning, PCR, RNA expression analysis) and instrumentation (centrifuges, phosphorimager, microplate-reader, digital microscope) to provide them with intense training and problem solving skills applicable to various career paths.

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