

AD \_\_\_\_\_

Award Number: DAMD17-00-1-0226

TITLE: How does nuclear organization maintain normal mammary phenotype?

PRINCIPAL INVESTIGATOR: Sophie A. Lelievre, DVM, Ph.D.

CONTRACTING ORGANIZATION: University of California at Berkeley  
Lawrence Berkeley National Laboratory  
Berkeley, California 94720

REPORT DATE: July 2001

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;  
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

20011127 124

# REPORT DOCUMENTATION PAGE

Form Approved  
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE July 2001	3. REPORT TYPE AND DATES COVERED Annual (1 Jul 00 - 31 Jul 01)
----------------------------------	-----------------------------	---

4. TITLE AND SUBTITLE  HOW DOES NUCLEAR ORGANIZATION MAINTAIN NORMAL MAMMARY PHENOTYPE?	5. FUNDING NUMBERS DAMD17-97-1-7276
---	--

6. AUTHOR(S) Sophie A. Lelievre, DVM, Ph.D
---

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of California at Berkeley Lawrence Berkeley National Laboratory Berkeley, California 94720 E-Mail:	8. PERFORMING ORGANIZATION REPORT NUMBER
--	--

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012	10. SPONSORING / MONITORING AGENCY REPORT NUMBER
---	--

11. SUPPLEMENTARY NOTES Report contains color
--

12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited	12b. DISTRIBUTION CODE
---	------------------------

13. ABSTRACT ( <i>Maximum 200 Words</i> )  Matrix protease-mediated degradation of the basement membrane (BM) surrounding breast epithelial units (acini) is associated with tumor progression. It is critical to understand the molecular mechanisms that underlie the maintenance of an intact BM in order to develop anti-cancer strategies. Using a human breast epithelial cell line (S1) that differentiate into acini in the presence of exogenous extracellular matrix, we have identified earlier a link between the nuclear organization of the protein NuMA and the regulation of matrix proteases. We have now engineered cDNA constructs coding for a putative histone-binding sequence (HBS) in NuMA that may play a role in the regulation of matrix proteases. As expected, over-expression of HBS restricted to the cytoplasm of S1 acinar cells doesn't affect BM maintenance. We are now studying the effect of intranuclear HBS on acini. We have also expressed and purified a poly-His-HBS fusion peptide to be used as bait to identify HBS binding partners. Most interestingly both the fusion peptide and the transgene expressed in S1 cells seem to oligomerize. This raises the possibility that HBS may act as either a monomer or a dimer depending on the differentiation status and this may regulate its interaction with potential binding partners.
---

14. SUBJECT TERMS Nuclear organization, NuMA, Acinar differentiation, Basement membrane, metalloproteases.	15. NUMBER OF PAGES 20
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited
---	--	---	---

## Table of Contents

<b>Cover.....</b>	<b>1</b>
<b>SF 298.....</b>	<b>2</b>
<b>Table of Contents.....</b>	<b>3</b>
<b>Introduction.....</b>	<b>4</b>
<b>Body.....</b>	<b>4</b>
<b>Key Research Accomplishments.....</b>	<b>14</b>
<b>Reportable Outcomes.....</b>	<b>14</b>
<b>Conclusions.....</b>	<b>14</b>
<b>References.....</b>	<b>15</b>
<b>Appendices.....</b>	<b>16</b>

## INTRODUCTION

The functional and structural epithelial units of breast tissue, referred to as acini and ducts, are maintained by the presence of a continuous basement membrane. The breakdown of the basement membrane by matrix proteases has been associated with loss of structural and functional differentiation and tumor progression, as illustrated by the transition between ductal carcinoma in situ and invasive carcinoma. Understanding how an intact basement membrane is maintained in normal epithelial structures should help develop new therapeutic tools to prevent cancer progression. We have recently demonstrated a link between the organization of the nuclear mitotic apparatus protein, NuMA, and the activation of matrix proteases and subsequent degradation of the basement membrane [Lelièvre et al., 1998]. We have hypothesized that NuMA organization induced by the formation of functional epithelial units is contingent upon its binding to other proteins to form multicomplexes, and that, in turn, the supramolecular organization of these multicomplexes is critical for the maintenance of the differentiation state. In order to decipher the molecular mechanisms that link NuMA organization to the maintenance of an intact basement membrane in breast epithelial units we have proposed to (1) investigate the role of specific N-terminal and C-terminal NuMA sequences we have identified as potential mediators of NuMA's functions (including matrix proteases activation) and (2) identify the binding partners of these sequences in conditions in which breast epithelial cells are and aren't differentiated into functional and structural units.

## BODY

Our working model is a non-malignant human mammary epithelial cell line HMT-3522 (S1) that can be induced to form functional acini surrounded by a complete endogenous basement membrane by growing S1 cells in the presence of an exogenous extracellular matrix (Matrigel<sup>BD</sup>) for 10 days [Petersen et al., 1992]. Using this system we started to investigate the role of NuMA sequences identified as potential effectors of NuMA functions in the establishment and maintenance of an endogenous basement membrane and search for the binding partners of these sequences as outlined in task 2 of the statement of work.

### *Task 2:* Search for NuMA binding partners. Year 1.

Our preliminary results showed that antibodies directed against the C-terminus of NuMA could induce the reorganization of NuMA from nuclear peripheral domains to a diffuse distribution throughout the nucleus, mimicking a pattern found in tumor cells [Lelièvre et al., 1998]. This reorganization was sufficient to trigger matrix protease activity and degradation of the basement membrane. NuMA protein consists of N-terminal globular (residues 1-207) and C-terminal (residues 1729-2115) domains separated by a discontinuous 1500 amino acid coiled-coil region. In collaboration with Dr. Saira Mian from the Lawrence Berkeley National Laboratory we have identified a histone-binding sequence at the C-terminus of NuMA (base pairs 6203-6450 corresponding to amino-acids 1983 to 2064) that also includes the bipartite nuclear localization signal-NLS (between base pairs 6203 and 6260) [Gueth-Hallonet et al., 1996] (figure 1A). This histone binding sequence belongs to the part of the protein that is cleaved in response to the loss of cell-basement membrane interaction [Lelièvre et al., submitted] and is absent in the fusion protein between NuMA and retinoic acid receptor that is thought to be

responsible for the tumorigenic phenotype in certain leukemia [Wells et al., 1997] (figure 1A). We have engineered two subsets of cDNA including the putative histone-binding domain by PCR: One contains the NLS and is based on the utilization of primers 1 and 3 (figure 1B), the other one is based on the utilization of primers 2 and 3 and is devoid of NLS sequence (figure 1B). The latter was intended to be used as a negative control for the nuclear effects of the C-terminal sequence transfected into non-malignant breast epithelial cells, since it shouldn't translocate to the cell nucleus, while construct 1-3 should. Primers 1 and 2 contain a restriction site for BAMHI and primer 3 contains a restriction site for HINDIII. In addition a FLAG tag was added at the N-terminus of each construct by including DNA FLAG sequence to primers 1 and 2. NuMA cDNA truncated for the N-terminus was used as a template for the PCR reaction (figure 9). c-DNA products obtained from primers 1-3 and 2-3 (minus NLS) showed the expected size around 1032 bp and 922 bp, respectively (figure 2). Each insert was subcloned at BAMHI and HINDIII sites in the pBluescript II SK vector plasmid that is used to maintain the stocks of C-terminal inserts. The DNA sequences of four clones per insert were assessed by automated sequencing of both forward and reverse sequences, using universal M13 and reverse primers.

C-terminus inserts were prepared for cell transfection by subcloning into pcDNA 3.1 plasmids and selecting for ampicillin resistance. Non-malignant S1-cells were transfected with the short construct (2-3) [NuMA-CT-noNLS] and are now being transfected with the construct 1-3 [NuMA-CT-withNLS] that contains the NLS. Transfected cells were selected for neomycin resistance with G418. S1-NuMA-CT-noNLS showed no significant alteration in their growth or capabilities to differentiate (including the establishment of cell-basement membrane polarization) in 3D culture as shown by immunostaining for a proliferation marker Ki-67 and polarization markers beta-catenin, alpha6 integrin and sialomucin (figure 3). This result is as expected since our hypothesis was that only the C-Terminus of NuMA allowed to go into the nucleus should affect the differentiation process and possibly basement membrane formation and maintenance. It seems therefore that the S1-NuMA-CT-noNLS transfected cells are a good negative control.

Expression of NuMA-CT-noNLS in S1 cells was assessed by western blot analysis of total protein extracts (Leamml procedure) ran within a 10% polyacrylamide gel and transferred onto a nitrocellulose membrane. Immunoblotting with anti-FLAG antibody revealed a doublet of 28 and 33 kDa (figure 4). This is higher than expected since the calculated molecular weight of NuMA-CT-noNLS with the FLAG tag is around 13.6 kDa. Signals with the same molecular weight were also detected by western blot and silver staining following immunoprecipitation with an anti-FLAG antibody (figure 5). The small number of tyrosine or serine/threonine residues that could be phosphorylated in the insert cannot explain the shift in molecular weight from 13.6 to around 30 kD. However, the shift in molecular weight could be due glycosylation of the C-terminus or its dimerization. Results obtained with purified NuMA-CT-noNLS peptide seem to confirm the hypothesis of dimerization, (see below); indeed, it has been shown in vitro that the C-terminus of NuMA can act as an initiator of oligomerization (Harborth et al., 1999). We will test this hypothesis by treating protein extracts with urea in order to disassemble the dimers and oligomers, and running proteins on a 15 % polyacrylamide gel, followed by short transfer and immunoblot with anti-FLAG antibody.

By using the FLAG tag we were hoping to be able to detect the peptide not only by western blot analysis using anti-FLAG antibodies but also by immunostaining. The results have been rather disappointing. The anti-FLAG antibody can stick non-specifically to cytoplasmic structures (probably the cytoskeleton as shown by immunostaining of non-transfected cells). Therefore it mainly produces a high background in our transfected cells. Although no staining can be found in the cell nucleus, which was expected since there is no NLS attached to FLAG-NuMA-CT~~no~~NLS, no significant distribution of FLAG-NuMA-CT~~no~~NLS can be observed in the cytoplasm. There was however an interesting pattern only seen in S1 cells both non-differentiated and differentiated in certain experiments, showing a concentration of staining at the cell membrane in contact with extracellular matrix components (figure 6). This needs to be confirmed with other antibodies. Since the success of NuMA-CT localization will be extremely important for some of our experiments we envision to use a new tag, V5-a 14 amino acid epitope, that has been shown to work well in the laboratory of our consultant Dr. Judy Campisi .

In order to detect potential ligands of NuMA C-terminus we have performed co-immunoprecipitation experiments with anti-FLAG antibodies in S1-NuMA-CT~~no~~NLS and T4-2-NuMA-CT~~no~~NLS cells. So far results have been analyzed in S1 cells by silver staining. A 95 kDa protein co-precipitated with NuMA-CT~~no~~NLS in the five experiments performed (figure 7). We are now investigating the possible candidates for this molecular weight. One possibility could be importin-beta, a protein that was found to interact with NuMA in different cell types and that is thought to regulate NuMA's capability to induce the formation of the mitotic spindle. Western blots for importin beta will be performed with the co-immunoprecipitates. These preliminary data were obtained with non-malignant S1 cells grown on 2D. Other co-immunoprecipitation experiments have been performed with differentiated S1 cells (3D culture) and will be analyzed soon. When we will perform the same type of experiments with cells transfected with NUMA-CT~~with~~NLS, we expect to find a subset of proteins that co-immunoprecipitate only when S1 cells are differentiated or a subset of proteins that do not co-immunoprecipitate anymore compared to non-differentiated conditions.

Another powerful way to analyze protein interactions with NuMA C-terminus histone-binding domain is to express and purify the peptide in vitro as a fusion protein and use it as bait. We have subcloned the cDNA for NuMA-CT~~no~~NLS in pRSET vector plasmid that contains a polyhistidine (6X) coding DNA sequence upstream of the cloning site (a similar process will be used for NuMA-CT~~with~~NLS). The choice of 6X His, instead of GST, for the fusion protein was based on the fact that our NuMA sequence is very small and GST is a big peptide that could impair the function and/or protein or DNA binding of NuMA-CT; whereas 6X His has been reported to allow complete function of small peptides. Polyhis-FLAG-NuMA-CT~~no~~NLS expression was induced in E. Coli (BL21-DE3-pLys) with 1 mM IPTG and the fusion peptide was purified using metal-exchange column (Xpress system from Invitrogen). Native NuMA-CT was run on polyacrylamide gel, transferred onto a nitrocellulose membrane and immunoblotted with anti-FLAG antibody. Four bands were detected, around 17, 30, 33, and 75 kDa (figure 8). The most intense signal corresponded to 31 kDa. This pattern resembles an oligomerization pattern. It includes a band at the expected molecular weight (14 kDa) and other bands corresponding to twice or four times this molecular weight. This observation

could explain why we also see a 29-31 kDa band in the transfected cells (see above). Experiments using UREA as a disassembly reagent and subsequent western blotting are underway. If what we are seeing is oligomerization we should see only one band around 14-17 kDa after UREA treatment of the purified fusion peptide. The fusion peptide will be used in affinity chromatography experiments and far Western blot experiments. Affinity chromatography experiments are underway: We are using 30 ug of purified peptide bound to Nickel sepharose. This mixture will be incubated with cell extracts from differentiated and non-differentiated cells. After washing, protein-protein binding will be released using 200 mM imidazole and proteins will be detected by silver staining on a polyacrylamide gel. However, if indeed NuMA-CT oligomerizes, it is possible that monomers of the peptide do not bind the same proteins as dimers (which is the form predominantly observed by western blot and silver staining). In this case we may have to perform chromatography affinity experiments and later on far western blot experiments with dimers and monomers of NuMA-CT in order to compare their binding capacity to other proteins.

Two other aspects of the experiments proposed in task 2 will be started soon. Production of the N-terminal sequence will start in the fall. Primers have already been designed and we will try using a V5 tag instead of FLAG. The onset of the two-hybrid experiment with the NuMACT-withNLS has been delayed until we know if NuMA-CT dimer can indeed bind proteins and until the cDNA libraries prepared in the laboratory of Mina Bissell (our collaborator) from non-malignant S1 and tumorigenic S1-derivatives (T4-2) mammary epithelial are ready. So far the libraries have been made in retroviral vector pBSF (pBABE) and their content for major regulators of mammary phenotypes is being assessed. The final set of experiments proposed in task 2 and scheduled to start at the end of the second year only, is contingent upon the production of the necessary tools (see task 1 below).

***Task 1:*** Production and analysis of acini expressing truncated forms of NuMA. Year 1. The main focus of this task is to produce transfected cells that lack either the C-terminus (CTT) or the N-terminus (NTT) of NuMA in order to create dominant negative effects of NuMA functions (figure 9). This task is complementary to task 2. There are two challenges in this task: We have (1) to manipulate large cDNA constructs (the inserts are above 6.8 kb) and (2) use them in an efficient TET-inducible system.

Plasmids containing CCT and NTT inserts were obtained from Dr. Compton (Dartmouth Medical School, Hanover, NH). However, these vector plasmids cannot be used for our transfection experiments. We have cut the inserts out of the plasmids with ECOR1 and isolated CTT further from the linearized plasmid by cutting with Sca1. NTT insert has been subcloned into the expression vector pCDNA3.1 that can accept big DNA fragments and a strain of bacteria, XL10-gold ultracompetent, that accepts large plasmids will now be transformed. This construct will then be used to transfect S1 cells. This system will be used to test if CTT or NTT forms of NuMA can induce matrix proteases activation and eventually tumorigenicity. It may however take a long time to get growing transfectant cells since the lack of terminal sequences in NuMA constructs may impair mitosis in nonmalignant cells; this is why we have proposed to also use a TET inducible system in which the expression of the transgene will be induced only upon completion of acinar differentiation when cells are growth arrested. We had planned originally to use a

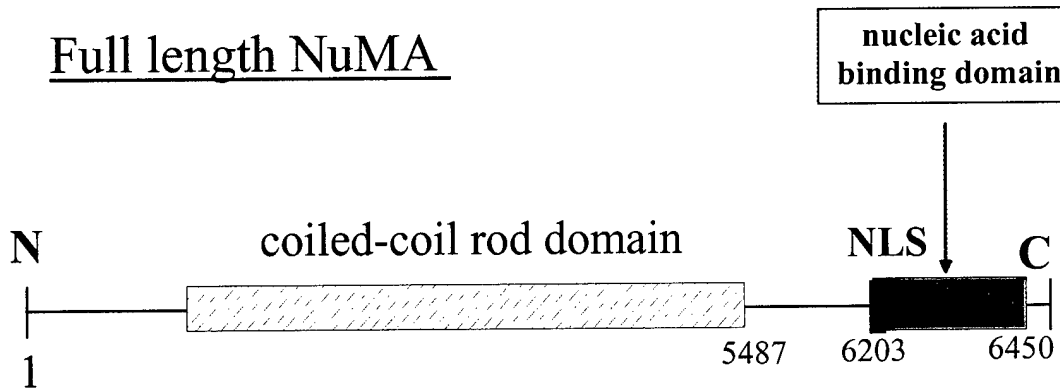
viral TET system, which would give high levels of incorporation of the transgene in our cell population. However, this system has not proven to be working efficiently, as tested by several of our colleagues including the laboratory of our consultant Dr. Judy Campisi. We have decided to use the original transfection-based system [a response plasmid pUDH 10-3 including the tet operator and a regulator plasmid pUHD15-1 including the tTA gene] made available to us by our colleague Dr Andrisani (Purdue University); S1-tTA cells are being selected. Once a stable cell line is obtained and examined for tTA expression via transient transfection with control plasmids, the S1-tTA cells that express reasonably low amounts of tTA will be transfected with CTT or NTT inserts contained in the pUDH 10-3 response plasmid.

The NTT construct does contain the NLS, therefore we will be able to test effects resulting from the presence of NTT in the cytoplasm and the nucleus. Whereas the CTT construct doesn't include an NLS anymore. We will have to engineer an NLS by including its DNA sequence at the C-terminus of the construct.

# NuMA C-Terminus

**A**

Full length NuMA

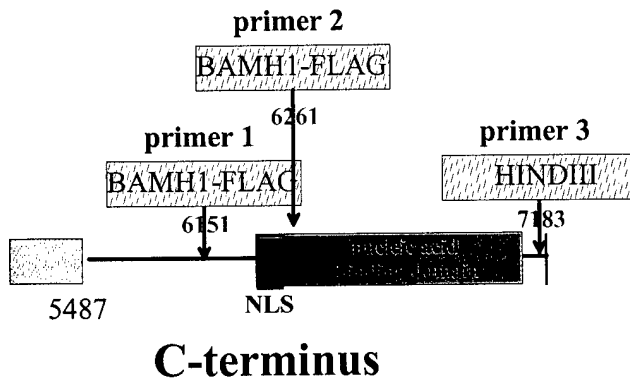


Fusion protein NuMA-RAR in leukemia



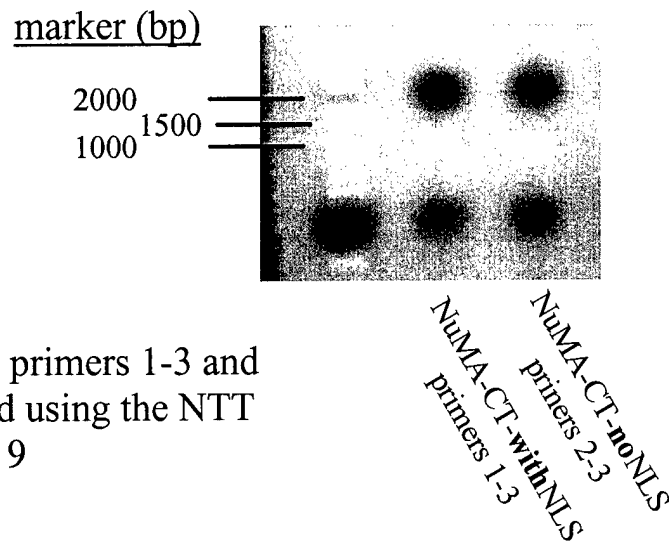
**B**

Primers used



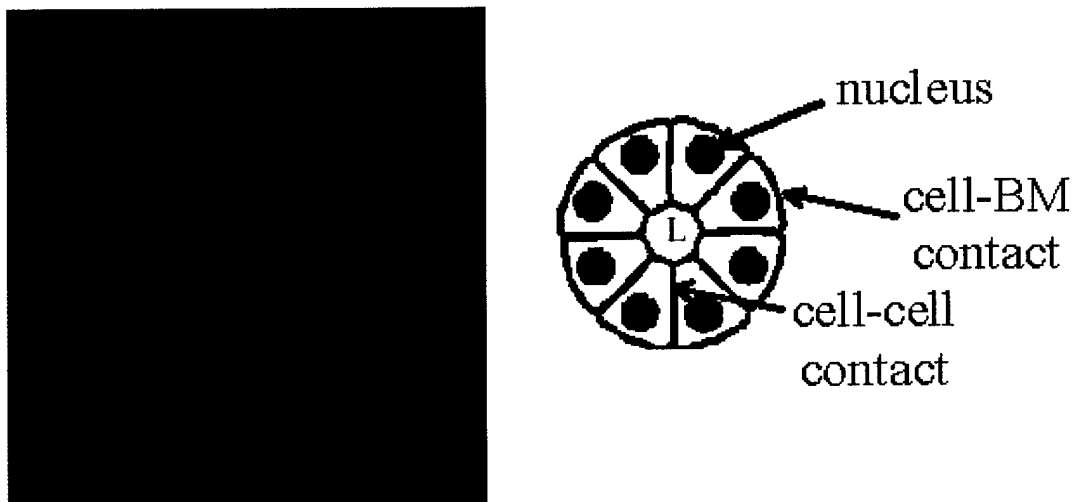
P.S.: numbers indicate base pair locations

Figure 2



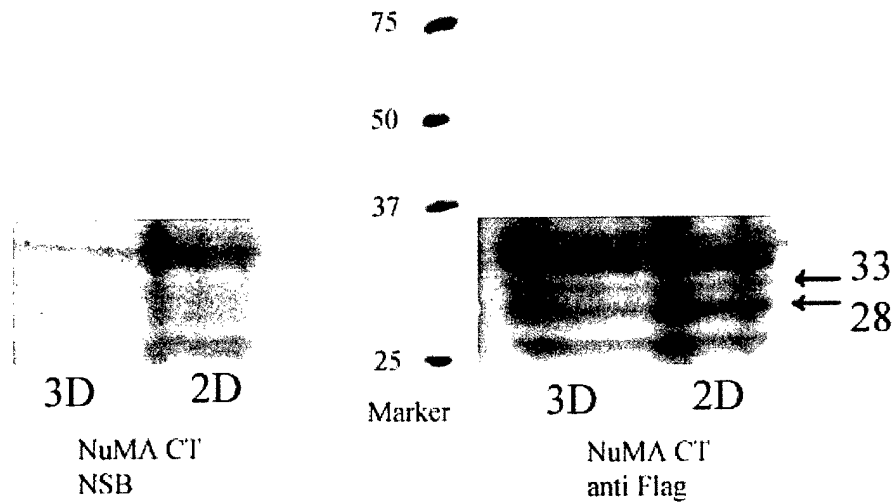
PCR products from primers 1-3 and 2-3 were engineered using the NTT template-see figure 9

Figure 3



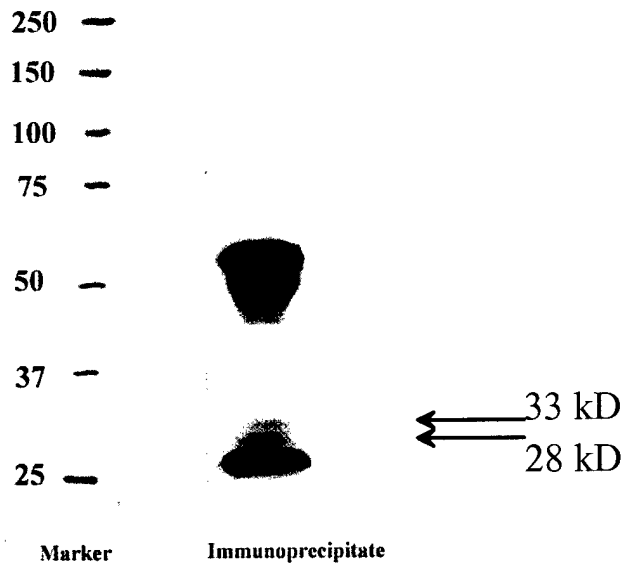
S1 cells transfected with NuMA-CT-noNLS differentiate well (acini formation) as shown by polarization markers beta-catenin (cell-cell contacts, in red) and alpha6 integrin (cell-basement membrane contact, in green)-Nuclei are stained with DAPI (in purple)

# Figure 4



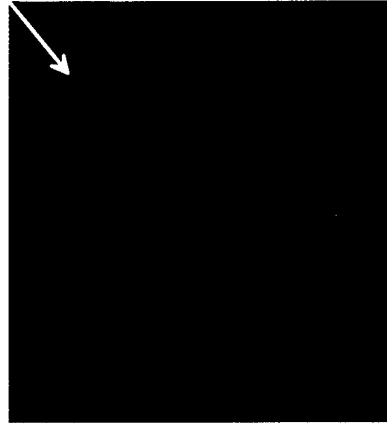
Western blots with anti-FLAG antibody: Total protein extracts were prepared from S1-NuMA-CTnoNLS cells cultured as a monolayer (2D culture) or differentiated into acini (3D culture). Transfer membranes were blotted with anti-Flag antibody (right) or secondary antibody only (left-non specific binding-NSB). A doublet of 28 and 33 kDa bands is found only in the sample incubated with anti-FLAG.

# Figure 5



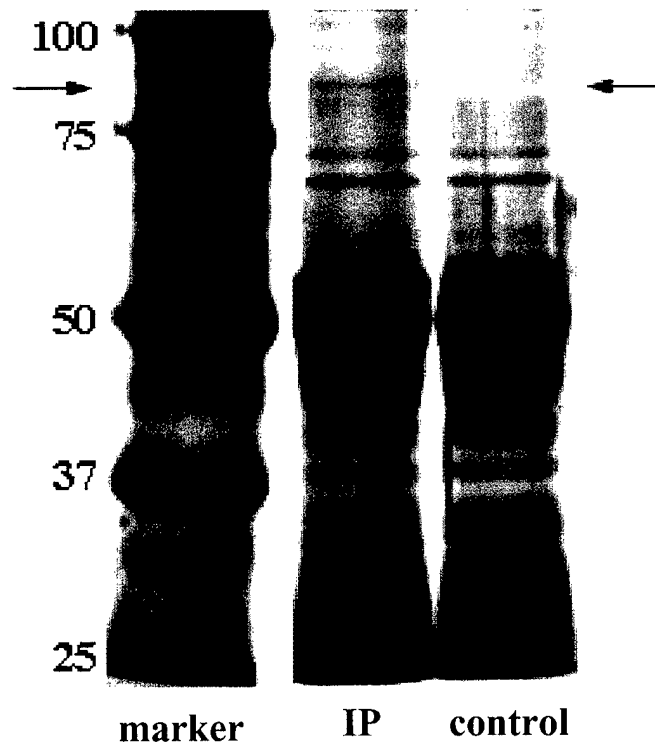
Immunoprecipitation with anti-Flag antibody from S1-NuMA-CTnoNLS cell extracts: a doublet is observed at 28-33 kD [immunoprecipitate]. The large signals above and below the doublet are non-specific (also found in the control-not shown here)

Figure 6



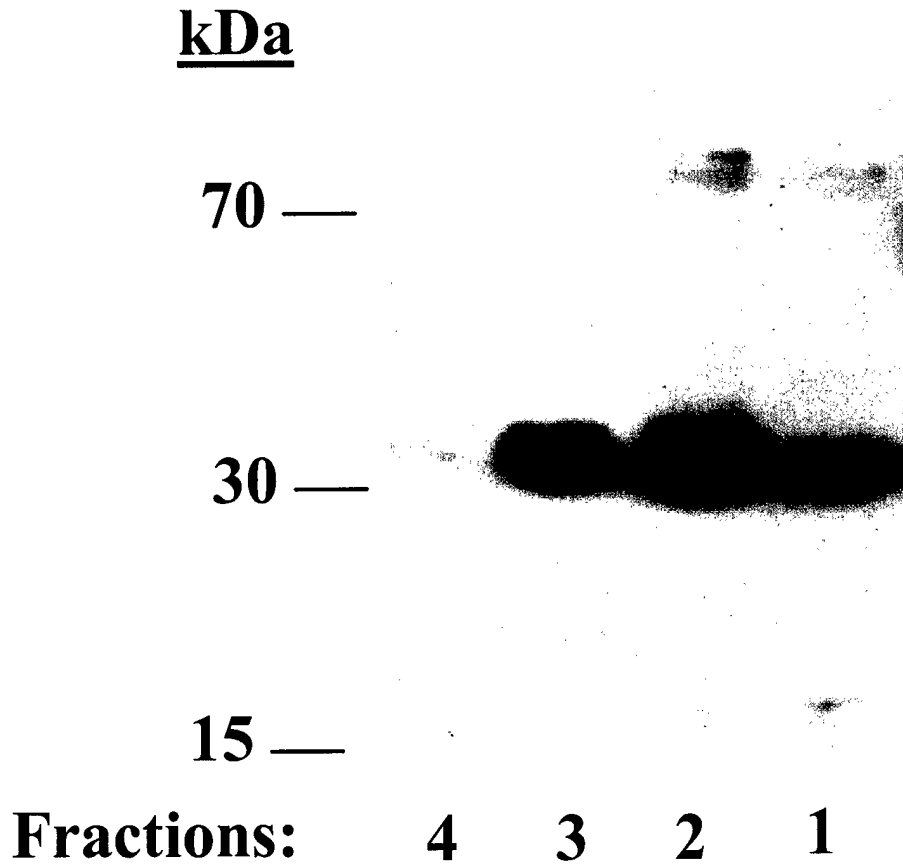
Immunostaining with anti-FLAG in S1-NUMA-CTnoNLS cells cultured as a monolayer. FLAG is in red, the arrow indicate an intense staining at the cell membrane. The actin network is the counterstain (in green) within the cytoplasm.

Figure 7



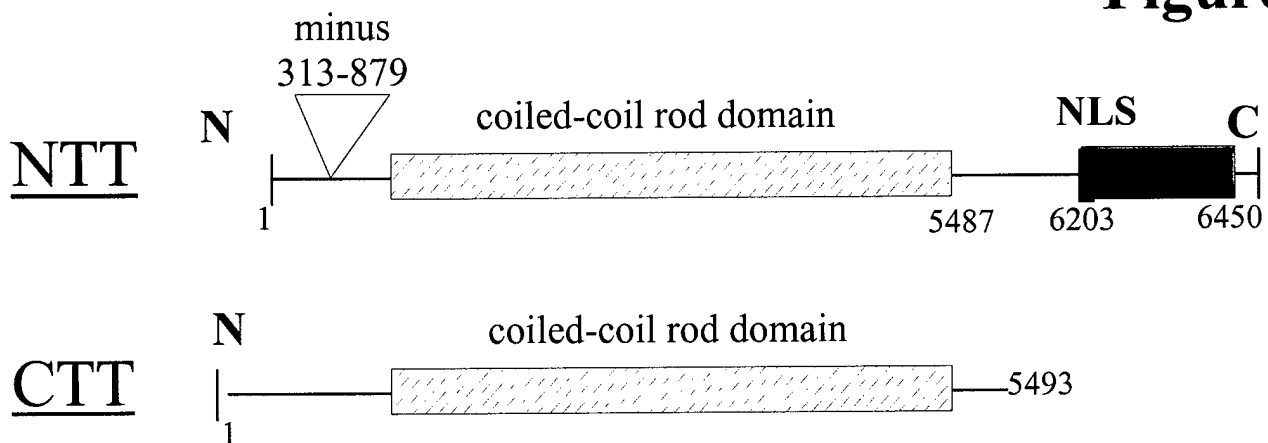
NuMA-CTnNLS was immunoprecipitated with antiFLAG antibodies (IP) or just secondary antibodies (control) from S1 transfected cells cultured as a monolayer. Precipitates were run in 10% polyacrylamide gel and the gel was silver stained. A 95 kD band is present only in the co-immunoprecipitate (IP) (see top arrow).

**Figure 8**



Fusion peptide poly-His-Flag-NuMA-CTnoNLS:  
Fractions collected after nickel sepharose purification of the fusion peptide. NuMA is mainly concentrated in fractions 1,2, and 3.  
Bands show up around 15, 33, and 75 kD after blotting with anti-FLAG antibody.

**Figure 9**



N-terminus (NTT) and C-terminus (CTT) truncated NuMA  
(numbers indicate base pair locations)

### **KEY RESEARCH ACCOMPLISHMENTS-year 1.**

- Engineering of cDNA coding for a potential histone-binding site at NuMA C-terminus.
- Production of different cDNA constructs: "NuMA-CT-noNLS" and "NuMA-CT-withNLS" inserted in pBSKII, pcDNA3.1 and pRSet plasmids.
- Production of polyHis-NuMA-CT fusion peptides in E. Coli.
- S1-NuMA-CT-noNLS transfected cells show no alteration of differentiation capability as expected. They can be used as negative control for S1-NuMA-CT-withNLS transfected cells.
- The putative histone-binding domain of NuMA produced in mammalian cells (transfected S1 cells) and the purified fusion peptide produced in E-Coli seem to be able to form homodimers and possibly oligomers.

### **REPORTABLE OUTCOMES- year 1.**

- 1) Development of cell lines: nonmalignant S1 cells expressing a potential histone binding sequence in NuMA C-terminus.
- 2) Production of Purified poly-His-fusion peptides for the putative histone-binding sequence of NuMA.
- 3) Employment: (a) The P.I., Dr. Sophie Lelièvre, was recruited as a Walther Assistant Professor in the Department of Basic Medical Sciences, School of Veterinary Medicine at Purdue University (West Lafayette, Indiana). The appointment is a joint appointment between the Department of Basic Medical Sciences and the Walther Cancer Center (see appendix). Only six of such positions are currently hold at Purdue University. This position officially started on October 2, 2000. Dr. Lelièvre laboratory now counts 7 members including technicians, postgraduate trainees and graduate students, and undergraduates. (b) As indicated in the budget, a research technician, Patricia Abad, has been recruited to work on the project.
- 4) Training: One senior undergraduate student successfully worked on part of the research project during the spring semester of 2001, as part of an exchange program for senior undergraduates between the University of Paris and Purdue University.

### **CONCLUSIONS**

During the first year of the research project we have engineered cDNA sequences corresponding to a putative histone-binding domain found at the C-terminus of NuMA. In addition, one type of sequence includes a nuclear localization signal (NLS), while the other type doesn't. This will enable us to differentiate between non-nuclear and nuclear-based effect of this putative histone-binding sequence in transfected cells. We have shown that non-malignant mammary epithelial cells transfected with the sequence devoid of NLS retain their differentiation capabilities. These cells are an important control for ongoing experiments in which non-malignant cells are being transfected with the histone-binding sequence that includes the NLS.

Our co-immunoprecipitation experiments in transfected cells have shown only one distinct additional band around 95 kDa compared to controls. A good candidate for this molecular weight is importin beta. If this hypothesis proves to be true, it will be important to check if importin beta still interacts with NuMA-CT upon differentiation when cells are growth-arrested, since the interaction between NuMA and importin beta has been mainly reported to regulate formation of the mitotic spindle.

An important observation is the fact that the putative histone binding sequence found in C-terminus of NuMA seems to be able to oligomerize (it forms mainly dimers); It may be of importance to understand how NuMA binding partners may interact with this sequence. For instance we could envision that depending on NuMA organization in the cell nucleus (which we have shown to be dynamic) the C-terminus may be either dimerized or not, and this may influence binding of potential partners and hence NuMA function.

## REFERENCES

- Lelièvre, S. A., V. M. Weaver, et al. (1998). "Tissue phenotype depends on reciprocal interactions between the extracellular matrix and the structural organization of the nucleus." *Proc Natl Acad Sci U S A* **95**(25): 14711-6.
- Petersen, O. W., L. Ronnov-Jessen, et al. (1992). "Interaction with basement membrane serves to rapidly distinguish growth and differentiation pattern of normal and malignant human breast epithelial cells." *Proc Natl Acad Sci U S A* **89**(19): 9064-8.
- Wells RA, Catzavelos C, Kamel-Reid S. (1997) Fusion of retinoic acid receptor alpha to NuMA, the nuclear mitotic apparatus protein, by a variant translocation in acute promyelocytic leukaemia. *Nat Genet.* 7(1): 109-13.
- Harborth, J., J. Wang, et al. (1999). Self assembly of NuMA: multiarm oligomers as structural units of a nuclear lattice. *Embo J* **18**: 1689-700.
- Gueth-Hallonet C, Weber K, Osborn M. (1996). NuMA: a bipartite nuclear location signal and other functional properties of the tail domains. *Exp Cell Res.*, 225:207-18

## APPENDICES

- 1) Walther/Purdue University Faculty position taken by the P.I.
- 2) C.V. of the P.I.

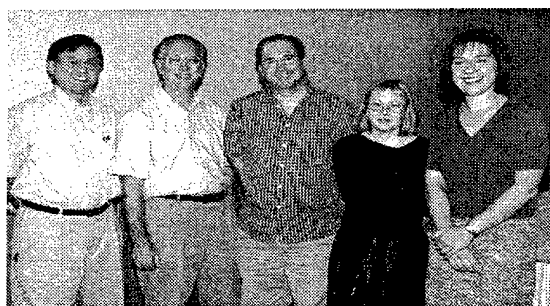
## WALTHER CANCER INSTITUTE PARTNERSHIP



Dr. Sophie A. Lelièvre

For the past thirteen years the Walther Cancer Institute has partnered with the Purdue Cancer Center in a joint pursuit to enhance cancer research in the State of Indiana. Located in Indianapolis, the Walther Cancer Institute was established by Dr. Joseph Walther as a nonprofit research institute dedicated to benefiting cancer patients through basic research. Since its inception the Walther Cancer Institute has been a driving force in cancer research throughout Indiana. The Institute significantly expanded its partnership with the Purdue Cancer Center in 1998 to stimulate the recruitment of seven cancer-focused scientists to the Center. In all, the Walther Cancer Institute will provide \$2.45 million over a five-year period to support the recruitment of these new Walther scientists to the Purdue Cancer Center. The partnership with the Walther Cancer Institute will allow the Center to significantly increase its cancer focus and recruit in areas of vital research interests to the Center.

During the 2000 academic year, the Center in close collaboration with the Department of Basic Medical Sciences in the School of Veterinary Science successfully recruited its sixth outstanding Walther Investigator to the Center. Dr. Sophie Lelièvre joined the Cell Growth and Differentiation program in the fall of 2000. Dr. Lelièvre trained at the Lawrence Berkely Laboratories with Dr. Mina Bissel. Dr. Lelièvre studies the changes in the architecture of the nucleus as a function of cell transformation.



Drs. Sakamuro, Bergstrom, Franklin, Hutchcroft, and Hrycyna

### Walther Investigators

**Donald Bergstrom, Ph.D.**

Walther Professor

Experimental Therapeutics and Diagnostics Program

**Jill Hutchcroft, Ph.D.**

Walther Assistant Professor

Cell Growth and Differentiation Program

**Dai Sakamuro, Ph.D.**

Walther Assistant Professor

Cell Growth and Differentiation Program

**David Franklin, Ph.D.**

Walther Assistant Professor

Cell Growth and Differentiation Program

**Christine Hrycyna, Ph.D.**

Walther Assistant Professor

Structural Biology Program

**SOPHIE LELIÈVRE, D.V.M., Ph.D.**

Assistant Professor of Basic Medical Sciences  
 Walther Cancer Center Investigator  
 Department of Basic Medical Sciences, 1246 Lynn Hall, Purdue University  
 West Lafayette, IN 47907-1246

Phone: (765) 496 7793  
 Fax: (765) 494 0781  
 E-mail: sal@vet.purdue.edu

**EDUCATION**

<b>Engineer</b>	Veterinary Sciences (highest honors; <i>Summa Cum Laude</i> 1985 and 1986) <i>University of Louvain, Belgium</i>	1984-1987
<b>Medical Degree</b>	Veterinary Medicine (honors) <i>University of Liège, Belgium</i>	1987-1990
<b>Master's Degree</b>	Molecular and Cellular Pharmacology (highest honors) <i>University of Paris VI, France</i>	1990-1991
<b>Ph.D.</b>	Molecular and Cellular Pharmacology (highest honors) <i>University of Paris VI, France</i>	1991-1994

**PROFESSIONAL POSITIONS**

<b>Veterinary Surgeon</b>	Pets Emergency Room, Paris District	1991-1995
<b>Predoctoral Fellow</b>	Gustave Roussy Cancer Institute (Villejuif, France) (topics: topoisomerases, anti-cancer pharmacology)	1991-1994
<b>Teaching assistant</b>	University of Paris (Embryology and Histology)	1991-1994
<b>Postdoctoral Fellow</b>	-Gustave Roussy Cancer Institute (Villejuif, France) and a 3-month training in Dr Kohwi-Shigematsu's laboratory (LJCRF, La Jolla, CA)(topics: resistance to topoisomerase inhibitors, metastatic phenotype, M.A.R., nuclear matrix)	1995
<b>Postdoctoral Fellow</b>	-Lawrence Berkeley National Laboratory	09/1995-1997
<b>Postdoctoral Scientist</b>	Dr Mina Bissell's laboratory (Berkeley, CA) (topics: extracellular matrix-nuclear structure interrelationship, regulation of gene expression in breast morphogenesis and tumorigenesis)	1997-1999
<b>Research Scientist</b>	Cell and Molecular Biology Dept. Lawrence Berkeley National Lab., Berkeley CA. (Topics: nuclear organization and gene expression, nuclear signaling)	1999-2000
<b>Assistant Professor and Walther Investigator</b>	Basic Medical Sciences Dept. Purdue University, West Lafayette IN. (Topics: nuclear organization in differentiation and cancer, nuclear signaling, nuclear structure and genomic instability)	2000-ongoing

**AWARDS AND HONORS**

**University of Louvain (Belgium)**, lifetime tuition exemption for outstanding student, 1985; **National Alexandre Joel Prize for young investigator**, 1995 (Association for Cancer Research, ARC, France); **National Prize for Fundamental Cancer Research/young investigator**, 1995 (French Society of Cancer and National Federation of Cancer Institutes, France); **Lawrence Berkeley National Laboratory Outstanding Performance Award**, 1998 (Lawrence Berkeley National Laboratory, Berkeley, CA); **Integrated Science Partnership Program Appreciation Award**, 1999 (Lawrence Berkeley National Laboratory, Berkeley, CA)

*Teaching Fellowship: University of Paris, 1991-1994.*

*Research Fellowships: French Ministry of Education and Research (France), graduate fellowship, 1991-1994; International Agency for Research on Cancer (IARC-WHO), postdoctoral fellowship, 1995-1996; Association for Cancer Research (ARC), complementary fellowship, 1996; Department Of Defense/USA-Breast Cancer Research Program (Postdoctoral Training grant) 1997-1999*

*Collaborative Research/Travel Fellowships: French Society of Cancer Travel Fellowship, 1995; Journal of Cell Science Travel Fellowship, 1997; Philippe Foundation Travel Fellowship, 1998.*

**PATENTS**

*"Utilization of nuclear structural proteins for targeted therapy and detection of proliferative and differentiation disorders"-applied for (IB-1454)*

## INVITED SEMINARS AND LECTURES

### Speaker at national and international meetings:

**"The solid-state signaling pathway from the extracellular matrix to the nuclear matrix: the critical role of 3D architecture at the cellular level"**, High resolution X-ray CMT Workshop (LBNL, Berkeley, CA), August 1996; **"Internal cell architecture-A new look"**, Advanced Light Source Users Meeting (LBNL, Berkeley, CA), October 1997; **"Global rearrangement of nuclear matrix-associated proteins when human mammary epithelial cells are cultured in 3-D: an analysis using confocal-, electron-, and soft x-ray microscopy"**, Keystone Symposium on Nuclear Matrix (Copper Mountain, CO; junior investigators workshop), April 1998; **"Nuclear structure, cell proliferation, and tissue morphogenesis"**, American Society for Cell Biology Meeting (San Francisco, CA), December 1998; **"Tissue architecture and gene expression: study of tissue matrix in three-dimensional models of cell culture"** and **"The non-chromatin structure of the nucleus or nuclear matrix: study of its interaction with the chromatin structure and its role in the regulation of gene expression"**, Biomathematics Summer School (Termoli, Italy), Mathematics in Cell Physiology and Proliferation, June 1999; **"Nuclear-directed signaling in mammary gland acini"**, Gordon Conference on Biological Structure and Gene Expression (Meriden, NH; short talk), August 1999; **"Nuclear organization in normal and malignant breast: NuMA is a marker of cell phenotype and a regulator of differentiation"**, Era of Hope DOD Breast Cancer Research Meeting (Atlanta, GA; platform talk), June 2000; **"Cell cycle regulation in higher order cell assemblies: the role of three-dimensional tissue architecture,"** Third International Congress of Nonlinear Analysts (Catania, Sicily) July 2000; **"Signal transduction and feedback signaling"**, **"Cellular transformation and genomic instability"**, **"Tumor progression: How in vitro models may help understand in vivo situations"**, ESMTB School, Biology and Mathematics of Cells: Physiology, Kinetics and Evolution, (Siguenza, Spain) June 2001.

### Seminars:

**"The nuclear matrix is an old concept still in its infancy"**, Gustave Roussy Cancer Institute, Villejuif, France (Dept. of Clinical and Molecular Pharmacology), September 1995; **"The solid-state pathway: a model for the regulation of gene expression"**, University of Paris XII, Creteil, France (CRRET Laboratory), June 1996; **"From the extracellular matrix to the nuclear matrix, the dynamic cellular architecture plays a role in the regulation of cellular behavior: a study of a model of mammary tumorigenesis"** Gustave Roussy Cancer Institute, Villejuif, France (Dept. of Clinical and Molecular Pharmacology), June 1996; **"Dynamic re-organization of nuclear architecture during tumorigenesis and tumor reversion"**, Harvard Children's Hospital, Boston (Dept. of Dr. Judah Folkman), April 1997; **"The role of cellular and tissue structure during tumorigenesis"**, Institute of Immunology, Munich, Germany (Dept. of Dr. G. Riethmüller), June 1997; **"Dynamic reciprocity between the extracellular matrix and the organization of the cell nucleus: a study of mammary epithelial cell morphogenesis"**, Institut de Génétique Moléculaire, Paris, France, June 1998; **"Interrelationships between the distribution of nuclear matrix proteins, chromatin structure and gene expression during mammary epithelial cells morphogenesis"**, CEA, Fontenay aux Roses, France, June 1998; **"Communication between the extracellular matrix and the nuclear structure in breast development and malignancy"**, Boston University Medical School, Dept of Biochemistry, February 1999; **"The role of nuclear organization in normal and malignant breast structures"**, California Pacific Medical Center Research Institute (San Francisco, CA), May 1999; **"Nuclear organization in normal and malignant breast"**, Division of Radiation and Cancer Biology, New England Medical Center, TUFTS University, Boston, MA); **"What is the link between nuclear architecture and the expression of malignancy?"**, Purdue University, Dept. of Basic Medical Sciences (West Lafayette, IN), March 2000; **"The organization of the cell nucleus in breast differentiation and tumorigenesis. A source for the development of novel anticancer strategies,"** Research Institute of Molecular Pathology, Vienna Biocenter (Boehringer-Ingelheim, Vienna, Austria), April 2000.

### Lectures:

**"Set up and use of 3 dimensional models of cell culture"**, M.I.T., Boston MA (invited by Dr. Sheldon Penman), November 1997; **"Use of complementary tools to image the nuclear structure of non-malignant and malignant cells"**, Lawrence Berkeley National Laboratory, California Science Project, January 1998; **"Cellular architecture and tissue phenotype: manipulations and visualization"**, Lawrence Berkeley National Laboratory, Integrated Science Partnership Project, July 1998; **"Multidisciplinary science for organ and tissue bioengineering"**, Lawrence Berkeley

National Laboratory, Integrated Science Partnership Project, July 1999; "From sheep to nucleus" Course MCB198, Maturing into a Research Scientist, University of Berkeley, September 1999 and March 2000; "The ascendance of Taxanes: Microtubules-targeted anti-neoplastic agents are 'back on the scene'" Purdue University, School of Veterinary Medicine (West Lafayette, IN), March 2000.

**PROFESSIONAL ACTIVITIES:** *Memberships to professional Societies:* Metastasis Research Society (1994-95); French Society of Cancer (1994-present); American Society for Cell Biology (1996-present); American Association for the Advancement of Science (1998-present).

*Reviewer for manuscripts:* Cancer Research, Journal of Cell Biology, carcinogenesis, DNA and Cell Biology (1996-present).

*Organization of scientific events:* co-organizer of the postdoctoral program "Challenges faced by PIs and postdoctoral fellows: Expanding career opportunities and initiating change" at the American Society for Cell Biology Meeting (December 1997); co-organizer of a Workshop Series on Effective Scientific Communication and Career (LBNL-PS and UC Berkeley Graduate Division 1998); organizing committee member for the International Society of Differentiation meeting (France, 2002).

*Committee member:* Student Delegate for the Belgian National Committee on the Education of Veterinary Medicine (1986-87); co-founder of LBNL Postdoctoral Society (President 1997-1998; Interim Chair of the Scientific Committee, March-September 1998); board member of the CF Lebrun High School Alumni Association (1999-present)

*Chair at scientific meetings:* Chair of the session on Cellular Organization, Signal Transduction and Cancer; "Biology and Mathematics of Cells: Physiology, Kinetics and Evolution" at the ESMTB meeting, Spain, 2001; co-chair of the session on "Nuclear compartmentalization in differentiation and cancer", International Society of Differentiation meeting, France, 2002.

## PUBLICATIONS

- K Bojanowski, S Lelièvre, J Markovits, J Couprie, A Jacquemin-Sablon and AK Larsen, "Suramin is an inhibitor of DNA topoisomerase II *in vitro* and in Chinese hamster fibrosarcoma cells". *Proc.Natl.Acad.Sci., USA*, 89:3025-3029, 1992.
- S Lelièvre and AK Larsen, "Development and characterization of suramin-resistant Chinese hamster fibrosarcoma cells: drug-dependent formation of multicellular spheroids and a greatly enhanced metastatic potential." *Cancer Res.*, 54: 3993-3997, 1994.
- S Lelièvre and AK Larsen, "L'acquisition de la résistance à la suramine dans les cellules de fibrosarcome de hamster chinois s'accompagne de modifications morphologiques et d'une augmentation de leurs capacités métastatiques". *Bull. Cancer*, 81: 903-905, 1994.
- S Lelièvre and AK Larsen, "Chronic *in vitro* suramin exposure leads to the development of drug resistant sublines which grow as three dimensional cultures and are highly invasive *in vivo*. Lack of growth factor involvement in the cytotoxic action of the drug." In "Novel approaches in anticancer drug design. Molecular modelling-New treatment strategies. *Contrib. Oncol.*, 49: 117-123, 1995, (WJ Zeller, D'Incalci M, and Newell DR, eds), Basel, Karger.
- S Lelièvre, Y Benchokroun, and AK Larsen, "Altered DNA topoisomerase I and II in suramin-resistant Chinese hamster fibrosarcoma cells." *Mol. Pharmacol.*, 47: 898-906, 1995.
- S Lelièvre, VM Weaver, and MJ Bissell, "Extracellular matrix signaling from the cellular membrane skeleton to the nuclear skeleton: a model of gene regulation in mammary epithelial cells." *Recent Progress in Hormone Research*, 51:417-432, 1996.
- S Lelièvre and Mina J. Bissell. "The solid-state signaling pathway from extracellular matrix to nuclear matrix: the critical role of three-dimensional architecture for functional differentiation." Proceedings of the 1996 Workshop on High Resolution Computed Microtomography (CMT), LBNL/UC, pp 85-96, 1997.
- S Lelièvre, VM Weaver, CA Larabell, and MJ Bissell, "Extracellular matrix and nuclear matrix interactions may regulate apoptosis and tissue-specific gene expression: a concept whose time has come." In *Advances in Molecular and Cell Biology: Cell Structure and Signaling*, (RH Getzenberg, ed), JAI Press Inc, Greenwich CT, Vol 24, pp: 1-55, 1997.
- SA Lelièvre, VM Weaver, JA Nickerson, CA Larabell, A Bhaumik, OW Petersen, and MJ Bissell. "Tissue phenotype depends on reciprocal interactions between extracellular matrix and the structural organization of the nucleus" *Proc. Natl. Acad. Sci. (USA)*, 95: 14711-14716, 1998.
- SA Lelièvre and MJ Bissell. "Communication between the cell membrane and the nucleus: the role of protein compartmentalization" 25<sup>th</sup> Anniversary Issue of *J. Cell. Biochem*, 30/31 suppl.: 250-263, 1998

- MJ Bissell, VM Weaver, SA Lelièvre, F Wang, OW Petersen, and KL Schmeichel, "Tissue structure, nuclear organization and gene expression in normal and malignant breast" *Cancer Res.(SUPPL)*, 59: 1757s-1764s, 1999.
- H-M Chen, KL Schmeichel, IS. Mian, SA Lelièvre, OW Petersen, and MJ Bissell. AZU-1: a candidate breast tumor suppressor and biomarker for tumorigenic reversion. *Mol.Biol.Cell*, 11: 1357-1367, 2000.
- SA Lelièvre, P. Pujuguet, and MJ Bissell. "Cell nucleus in context." *Crit. Rev. Eukar. Gene Expression*, 10: 13-20, 2000.
- W Meyer-Ilse, D Hamamoto, A Nair, SA. LeLièvre, G Denbeaux, L Johnson, A Lucero, D Yager, and CA. Larabell. "High Resolution Protein Localization Using Soft X-ray Microscopy." *J. Microscopy*, 201: 395-403,2001
- C Ortiz de Solorzano, R. Malladi, SA Lelièvre, and SJ Lockett. "Segmentation of nuclei and cells using membrane related protein markers." *J. Microscopy* , 201: 404-15, 2001
- SK Muthuswamy, D Li, SA Lelièvre, MJ Bissell, and J Brugge. " ErbB2 but not ErbB1 reinitiate proliferation and induce repopulation of the lumina in growth-arrested epithelial acini" *Nature (Cell Biology)* (In press).
- VM Weaver, SA Lelièvre, JN Lakins, J Jones, F Giancotti, Z. Werb, and MJ Bissell. "Beta4-integrins induce polarity and apoptosis-resistance in mammary tissue structures". (Submitted)
- SA Lelièvre, VM Weaver, CA. Larabell, and MJ Bissell. "Inside-out signaling by NuMA connects basement membrane signaling and apoptosis". (submitted)
- SA Lelièvre, SH Kim, P Kaminker, OW Petersen, P Chung, MJ Bissell, and J Campisi. "Clustering of telomere-associated proteins regulates breast acinar differentiation". (submitted).
- P Abad, A Viron, E Puvion, JA Nickerson, MJ Bissell, and SA Lelièvre. NuMA shuttles between different subcellular locations in differentiated and tumor breast epithelial cells. (In preparation).