Agència de Gestió d'Ajuts Universitaris i de Recerca

AGAUR

# Memòria justificativa de recerca de les convocatòries BCC, BE, BP, CTP-AIRE, INEFC i PIV

**La memòria justificativa consta de les dues parts que venen a continuació:**
**1.- Dades bàsiques i resums**
**2.- Memòria del treball (informe científic)**

Tots els camps són obligatoris

## 1.- Dades bàsiques i resums

### Nom de la convocatòria

**BP**

**Llegenda per a les convocatòries:**

| | |
|---|---|
| BCC | Convocatòria de beques per a joves membres de comunitats catalanes a l'exterior |
| BE | Beques per a estades per a la recerca fora de Catalunya |
| BP | Convocatòria d'ajuts postdoctorals dins del programa Beatriu de Pinós |
| CTP-AIRE | Ajuts per accions de cooperació en el marc de la comunitat de treball dels Pirineus.<br>Ajuts de mobilitat de personal investigador. |
| INEFC | Beques predoctorals i de col·laboració, dins de l'àmbit de l'educació física i l'esport i les ciències aplicades a l'esport |
| PIV | Beques de recerca per a professors i investigadors visitants a Catalunya |

**Títol del projecte:** ha de sintetitzar la temàtica científica del vostre document.

Biophysical characterization of tubulin tyrosine ligase-like proteins on microtubule dynamics
Elucidating the Mechanism of Microtubule Severing by Katanin
Fabrication of hydrogels with steep stiffness gradients for studying cell mechanical response

### Dades de l'investigador o beneficiari


| Nom | Cognoms |
|---|---|
| Raimon | Sunyer i Borrell |

Correu electrònic
raimonsun@gmail.com


### Dades del centre d'origen

National Institutes of Health

### Número d'expedient

2009 BP-A 00224

**Paraules clau:** cal que esmenteu cinc conceptes que defineixin el contingut de la vostra memòria.

Microtubules, Tubulin, Tubulin Tyrosine Ligase, mechanostransdiction, hydrogels,

### Data de presentació de la justificació

Raimon Sunyer i Borrell

Nom i cognoms i signatura
del/de la investigador/a

Ralph Nossal

Vist i plau del/de la responsable de la
sol·licitud

**Resum del projecte:** cal adjuntar dos resums del document, l'un en anglès i l'altre en la llengua del document, on s'esmenti la durada de l'acció

**Resum en la llengua del projecte** (màxim 300 paraules)

El document ha estat redactat en angles

**Resum en anglès** (màxim 300 paraules)

**Biophysical characterization of tubulin tyrosine ligase-like proteins on microtubule dynamics**
Microtubules (and their subunit tubulin dimer) are important components of the cytoskeleton and carry out a variety of essential functions. Posttranslational modifications (PTM) of tubulin mark subpopulations of microtubules and selectively affect downstream microtubule based functions. The members of the tubulin tyrosine ligase like (TTLL) family are proteins that trigger different PTM on tubulin and microtubules. How TTLL triggered PTM regulate microtubule dynamics remains obscure. The aim of this part of my postdoctoral research was to explore how the activity of the TTLL family affects microtubule dynamics. In the first part of the project, I obtained the DNA plasmids that encode GFP tagged TTL, TTLL3, TTLL4, TTLL7, TTLL11. I successfully transfected living cells with them. Then, I designed an assay to measure microtubule dynamics in living cells. Finally I observed how cells overexpressing these proteins affect microtubule dynamics.

**Fabrication of hydrogels with steep gradient of stiffness for screening mechanotransduction mechanisms**
Many fundamental cell processes, such as angiogenesis, neurogenesis and cancer metastasis are modulated by the ability of cells to sense extracellular matrix stiffness by a process called mechanotransduction. In different tissues, extracellular matrix stiffness varies over several orders of magnitude from cartilage as stiff as 100kPa to soft brain tissue as compliant as 0.2kPa. Thus, the availability of matrix substrates having well-defined stiffness profiles can be of great importance in biophysical studies of how cells interact with their environment. Existing methods to fabricate matrices have either insufficient resolution or are costly to implement. Therefore, I developed a new method to fabricate matrices with a steep gradient of stiffness suitable to be used for screening mechanotransduction mechanisms. This fabrication method presents a wider range of rigidity and gradient than current methods. This work has been published in the journal PLoS ONE.

I carried out this research from July 1st 2010 until July 1st 2012.

**Resum en anglès** (màxim 300 paraules) – continuació -.

**2.- Memòria del treball** (informe científic sense limitació de paraules). Pot incloure altres fitxers de qualsevol mena, no més grans de 10 MB cadascun d'ells.

Thanks to the Beatriu de Pinos Program, I have been doing my postdoctoral research at the National Institutes of Health (NIH) located in Bethesda, MD USA. The NIH is an agency of the United States Department of Health and Human Services and is the primary agency of the United States government responsible for biomedical research. In addition to provide funding (USD $31.2 billion) and services for the scientific community (e.g. Pub Med), NIH also carries out an intense research activity with more than 6,000 scientists in its own laboratories working on a broad variety of biomedical disciplines. NIH unique environment has allowed me to complete a highly interdisciplinary research that in a more conventional institution would has been too difficult to carry out. Furthermore, at NIH I have complemented my bioengineering skills with advanced techniques of cell biology and biochemistry. I have been working at NIH since early 2010 (3 years by the end of 2012).

## Biophysical characterization of tubulin tyrosin ligase (TTL) function in cells

Microtubules are an important component of the cytoskeleton and carry out a variety of essential functions. This diversity of roles raises the question of how individual microtubules are assigned to these specific functions. In other words, what distinguishes a spindle microtubule from one that is part of a cilium? Recent work has shown that posttranslational modifications (PTM) of the tubulin building blocks mark subpopulations of microtubules and selectively affect downstream microtubule based functions. The first discovered protein that triggers PTM is the tubulin tyrosine ligase (TTL). TTL catalyzes the post-translational C-terminal tyrosination of $\alpha$-tubulin. Tyrosination regulates recruitment of microtubule-interacting proteins. Its loss causes morphogenic abnormalities and is associated with cancers of poor prognosis. The TTL belongs to the tyrosine ligase like (TTLL) protein family. The members of this family are proteins that trigger different PTM on tubulin and microtubules. The aim of this part of my postdoctoral research has been to obtain a DNA plasmid that encodes GFP-tagged TTL and TTLL and design an assay to characterize its interaction with microtubules in living cells.

*Construction of DNA plasmids that encode GFP tagged TTL, TTLL3, TTLL4, TTLL7, TTLL11 and live cell transfection*

In the first part of the study, I learned how to clone genes, tag them with a GFP molecule (C- or N-terminal) and inset them on a destination vector with a CMV promoter. Using this approach I obtained recombinant DNA for the enzymes TTL, TTLL3, TTLL4, TTLL7 and TTLL11. Plasmid transfection of human osteosarcoma cells (U2-OS cell line) with electroporation revealed that transfected cells expressed GFP. I used western blot analysis to confirm that TTLs levels were overexpressed on transfected cells. Immonufluorescence against post translational modified microtubules (glutamilated tubulin for TTL and long and short chains of polyglutamine for the rest of the TTLL family) revealed that TTLs enzymes were functional within the cell. Finally, I also cloned a TTL mutant (E331Q) defective in tubulin tyrosination but not in tubulin binding.

*Design an assay to measure microtubule dynamics in living cells*

To visualize microtubule dynamics on living cells we fluorescently tagged plus end–binding protein EB3 of human osteosarcoma cells. EB3 marks the ends of growing microtubules and can be used to determine the growth rates of microtubules from time-lapse image series. We obtained a stable cell line expressing EB3-GFP by selecting U2-OS cells with the FACS sorting machine and geneticin antibiotic. Microtubule dynamics was quantified by computationally tracking a large number of microtubule growth events (visualized as individual EB3 comets). Then, we modified a recently developed method for spatiotemporal grouping of growth tracks to extract additional parameters of microtubule dynamic instability such as microtubule growth rate, shrinking probability and shrinking rate. I also implemented a MatLab application that correlates the expression levels of GFP-TTL/TTLL with spatiotemporal variation of microtubule dynamics parameters within individual cells.

*Measure of TTL and TTLLs effect on microtubule dynamics*

We analyzed microtubule growth rates in U2OS cells while manipulating TTL and TTLLs concentrations. Overexpression of GFP-TTL at low concentrations reduced the speed of microtubule growth (average growth rate was $13.04 \pm 0.07$ μm $min^{-1}$ (mean ± s.d.; n = 4,225 tracks from four cells) for wild type and $10.86 \pm 0.08$ μm $min^{-1}$ for cells expressing GFP-TTL (n = 3,787 from four cells). Because tyrosination can influence the recruitment of Cap-Gly domain containing plus end–binding proteins that affect microtubule dynamics, we also investigated the effects of a TTL mutant (E331Q) defective in tubulin tyrosination but not in tubulin binding. Live cell imaging with this mutant showed that it had a similar effect on microtubule growth rates to wild-type TTL (average growth rate was $10.24 \pm 0.12$ μm $min^{-1}$ (n = 5,222 from five cells); indicating that the reduction in microtubule growth rates was not due to increased tubulin tyrosination.

When also analyzed changes in microtubule dynamics on cells transfected with TTLL3, TTLL4, TTLL7, TTLL11. However, Overexpression of these proteins on U2-OS cells did not significantly change microtubule growth rate, shrinkage probability or shrinkage speed. We are currently preparing a manuscript from this study.

## Elucidating the Mechanism of Microtubule Severing by Katanin

During this project I also implemented a magnetic tweezers setup capable of exerting and detecting small forces. Magnetic tweezers consist of an electromagnet with a ferromagnetic core in the form of a sharp needle. By means of a micromanipulator, this ferromagnetic core can be moved and placed near the sample. A magnetic gradient is generated by applying current to the electromagnet. This magnetic gradient is able to pull paramagnetic beads with controlled force. The magnitude of this force depends on the magnetic gradient as well as the distance between the needle tip and the beads, and can be calibrated by pulling beads suspended in fluids of known viscosity. The device was designed to exert

significant forces at the molecular level, ranging from ~100 pN to ~ 10 nN. The magnetic tweezers were controlled with a LabView application developed during my PhD. This LabView application was able to track the position of the magnetic beads with nanometer resolution.

With this magnetic tweezers set up we have been studying the mechanisms of action of katanin. We are currently overcoming some technical difficulties that do not allow us to visualize microtubule severing with enough resolution.

### Fabrication of hydrogels with steep gradient of stiffness for screening mechanotransduction mechanisms

In addition to the studies mentioned above, in my postdoctoral training I have also worked on a project that of interest for the laboratory of Dr. Trepat at the Institute for the Bioengineering of Catalonia, where I am currently presenting a Beatriu de Pinos B application. Dr. Trepat is interested in screening for proteins involved in mechanotransduction pathways. Many fundamental cell processes, such as angiogenesis, neurogenesis and cancer metastasis are thought to be modulated by the ability of cells to sense the extracellular matrix stiffness in a process called mechano-transduction. In different tissues, extracellular matrix stiffness varies over several orders of magnitude from cartilage as stiff as 100 kPa to soft brain tissue as compliant as 0.2 kPa. Moreover, the surfaces upon which cells move are heterogeneous. Thus, the availability of matrix substrates having well-defined stiffness profiles can be of great importance in biophysical studies of how cells might interact with their environment. Existing methods to fabricate matrices have either insufficient resolution or are costly to implement. Therefore, the aim of this work is to develop a method to fabricate matrices with a steep gradient of compliance (as much as 240 kPa across a 2 mm gel) suitable to be used in a gene screening assays.

Therefore, we developed a novel method to fabricate bio-compatible hydrogels with a well defined and linear stiffness gradient. This method, involving the photopolymerization of films by uncovering an acrylamide/bis-acrylamide solution with an opaque mask, can be easily implemented with common lab equipment. Young's modulus characterization across the gel reveals that this technique produces stiffness gradients up to 120 kPa/mm, 3-fold steeper than gradients produced by microfluidic techniques. The stiffness range of these hydrogels (from 1 kPa to 240 kPa) is 900% larger than the range produced by microfluidic generators. Hydrogels with milder gradients and stiffness ranges can easily be produced as well. The hydrogels can be covalently functionalized with proteins that promote cell adhesion. Ligand density is independent of the stiffness gradient.

The spreading of cells attached to gradient stiffness hydrogels linearly correlates with hydrogel stiffness indicating that this technique effectively modifies the mechanical environment of living cells. We are currently writing a manuscript to describe the fabrication process of these gradient stiffness hydrogels.

This work has been published in the journal PLoS ONE (R. Sunyer, AJ Jin, DL Sackett and R. Nossal, Fabrication of hydrogels with steep stiffness gradients for studying cell mechanical response, PloS ONE, in press).

## Attendance to congresses and conferences

- Sunyer R, Jin, A, Sackett, DL and Nossal R. Fabrication of hydrogels with gradient of compliance: application to cell mechanotaxis and durotaxis. Biophysical Society Meeting, San Diego, CA (USA). (February 24$^{th}$- March 1$^{st}$ 2012). Poster

## Publications

- Sunyer R, Jin AL, Sackett DL and Nossal R. Fabrication of hydrogels with steep stiffness gradients for studying cell mechanical response. PLoS ONE, *in press*

- Sunyer R. Effect of tubulin tyrosine ligase (TTL) on microtubule dynamics. *Manuscript in preparation*