How to write a research proposal

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Before writing a proposal, you should know

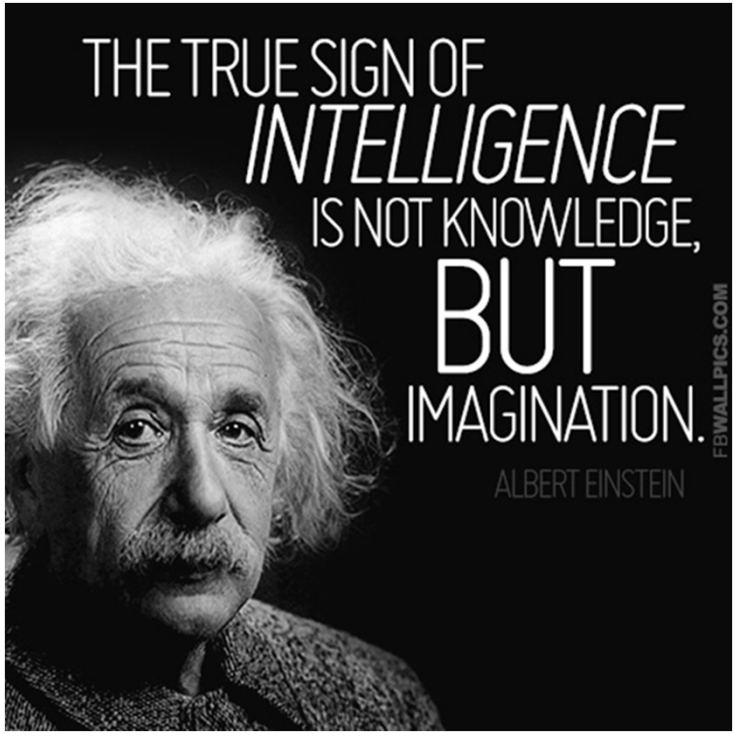
What do you plan to do?

Why do you want to do it?

How are you going to accomplish it?

Five Golden Rules for Getting a Grant

- **Rule 1: Be novel but not too novel**
- **Rule 2: Simple but not shallow**



- **Rule 3: Obey the Three Cs—**
  Concise, Clear, and Complete

- **Rule 4: Include the appropriate background and preliminary data as required—**
  Good quality, Clear legend, Impressive, and Supporting

- **Rule 5: Keep the main theme.**
  Consistent NOT divergent

Writing a proposal

There is no fixed formula for writing a proposal.

However, your challenge is to **convince** members of the scientific community that you

- have identified a scientific question
- have a theoretical background and a methodical approach to answer the question
- within a realistic time frame and at reasonable expenses.

Research question

Your research question **is the most critical part** of your research proposal—it defines the proposal, it guides your arguments and inquiry, and it provokes the interests of the reviewer.

- State and justify your questions/objectives clearly ("because it is interesting" is not enough!)
- Ask yourself why this research should be funded/why this research is important and/or timely
- How will the research benefit the society or contribute to the research community?

Suggested structure for a research proposal

- Title and abstract
- Background information/brief summary of existing literature (3)
- Hypothesis, objectives, and significance (1)
- Preliminary results (3~5)
- Strategies, experimental design, and methodology (6~8)
- Anticipated results (1)
- Competence of the Investigators
- Conditions of the Research Environment
- Ethical considerations

(2)

- Time table and Budget
- References

Title:

It should be concise and descriptive.

Abstract:

It is a brief summary of approximately 300-500 words.

It should include the background, the research question, the rationale, the preliminary results, the hypothesis (if any), the goal, the method (platform/new technology), the expected outcomes and the impacts.

科技部專題研究計畫申請書

申請條碼：105WFA0550100

一、基本資料：

計畫類別（單選）	一般研究計畫				
研究型別	個別型				
計畫歸屬	生科司				
申請機構/系所（單位）	國立中興大學生命科學系（所）				
本計畫主持人姓名	陳鴻震	職稱	教授且兼任生命科學院院長	身分證號碼	A12305****
本計畫名稱 中文	探討Adducin-1在維持紡錘體端點完整性的角色				
本計畫名稱 英文	Study on the role of Adducin-1 in maintenance of spindle pole integrity				
整合型總計畫名稱					
整合型總計畫主持人				身分證號碼	
全程執行期限	自民國 105 年 08 月 01 日起至民國 108 年 07 月 31 日				
研究學門	學門代碼	學門名稱			
	B10B002	醫學生化及分子生物			
研究性質	■純基礎研究 □導向性基礎研究 □應用研究 □技術發展				

Abstract

Keywords: mitosis, spindle, spindle pole, centrosome, centriole splitting, Adducin, TPX2

1Bipolar mitotic spindle formation is essential for faithful chromosome segregation during mitosis. Defects in centrosome number and structural organization can lead to multipolar spindle, which is a hallmark of tumor cells and often associates with chromosomal instability. 2Adducin-1 (ADD1) is an actin-binding protein that is important for the stabilization of the membrane cortical cytoskeleton and cell-cell adhesion. In 2014, we reported a novel function for ADD1 in mitotic spindle assembly through its interaction with myosin-X. ADD1 depletion causes not only distorted and elongated spindles, but also multipolar spindles. In this proposal, we set out to explore the possible role of ADD1 in mitotic centrosomes. Our preliminary findings indicate that ADD1 is phosphorylated at S726 during mitosis and the S726-phosphorylated ADD1 is specifically localized at mitotic centrosomes. In addition, we found that the multipolar spindle caused by ADD1 depletion result from centriole splitting. Importantly, we identified TPX2 as an ADD1-binding partner in mitosis. TPX2 is a multifunctional microtubule-associated protein, which has been known to be important for both spindle assembly and spindle pole integrity. Based on our preliminary findings, we **hypothesize** that ADD1-TPX2 interaction may be crucial for spindle pole integrity. The **goal** of this proposal is to understand the role of ADD1 in the maintenance of spindle pole integrity. To reach this goal, six **specific aims** are proposed:

Aim 1. To examine the sub-compartment localization of S726-phosphorylated ADD1 in centrosomes. (1st year)

Aim 2. To establish the significance of ADD1 phosphorylation at S726 in spindle pole integrity. (1st year)

Aim 3. To examine the regulation of ADD1 S726 phosphorylation in mitosis. (2nd year)

Aim 4. To characterize the interaction between ADD1 and TPX2. (2nd year)

Aim 5. To establish the significance of the ADD1-TPX2 interaction in spindle pole integrity. (3rd year)

Aim 6. To examine the mechanism by which the ADD1-TPX2 interaction contributes to spindle pole integrity. (3rd year)

B

R

Q

P

Significance:

Proper control of mitosis is necessary for cells to maintain their genetic integrity during cell division. How cells ensure bipolar spindle assembly and spindle pole integrity has been a fundamental question in biology. This proposal aims to study the mechanism of how centrosomes keep their integrity in mitosis by focusing on ADD1 and its interaction with TPX2. The achievement of this proposal will enhance our understanding to the molecular mechanism for the control of spindle pole integrity. In addition, the achievement of this study will provide the first example to show that an actin-binding protein (such as ADD1) is involved in centrosome's structure and function. I believe that the results of this study will be published in the first-tier journal and have significant impact to the society of cell biology.

Suggested format for an Introduction

- Introduce the area of research
- Review key publications
- Identify any gap in the knowledge or questions which have to be answered
- Your hypotheses
- Your objectives
- How is your research beneficial

How will you achieve the research aims?

Strategies, Experimental design, and Method

It is important to present the proposed research methodology (e.g. techniques, sample size, target populations, species choice, equipment and data analysis) and explain why it is the most appropriate methodology to effectively answer the research question.

Aim 1. To examine the sub-compartment localization of S726-phosphorylated ADD1 in centrosomes.

Our preliminary results indicate that S726-phosphorylated ADD1 is likely to be a component of pericentriolar materials (PCM) (Fig. 2-4). Recently, PCM was found to be assembled as a cartwheel-like structure in the centrosome (Lawo *et al*., 2012). Advanced microscopy with nanoscale resolution will be needed to locate the sub-compartment localization of ADD1 relative to other PCM proteins. The information about ADD1's sub-compartment in centrosomes will provide a clue for its intimate partners in PCM, which may function as a scaffold to "hold" centriole pair and therefore maintain the centrosome integrity. In addition, it is not clear whether S726 phosphorylation is necessary for ADD1 to localize to centrosomes. It is possible that ADD1 is first recruited to the centrosome by PCM protein(s) and then phosphorylated by mitotic kinase(s) within the centrosome. We have previously found that ADD1 binds to Myo10 (Chan *et al*., 2014). The role of Myo10 in the recruitment of ADD1 to the spindle pole will be examined as well.

Aim 1-1: To examine the sub-compartment localization of S726-phosphorylated ADD1 in centrosomes by super-resolution microscopy.

(1) Structured illumination microscopy (SIM)

For SIM, the sample is illuminated with a grid pattern, which is then shifted while multiple images are acquired. A super-resolution image is then calculated computationally from the data. SIM can achieve resolution of ~120 nm in lateral direction (Schermelleh *et al*., 2008). Layered organization of PCM proteins has been revealed by SIM (Lawo *et al*., 2012). We will stain for ADD1 pS726 and other PCM proteins such as pericentrin, γ-tubulin and NEDD1 to visualize the hierarchical structure of S726 phosphorylated-ADD1 in the PCM.

- The Imaging Core at the Institute of Molecular Biology, Academia Sinica, can provide a service for SIM (ZEISS ELYRA PS.1).

(2) Stochastic optical reconstruction microscopy (STORM)

To observe more detailed structure features of S726 phosphorylated-ADD1, we will employ STORM microscopy, a single molecule localization technique with a resolution up to 10 nm in the X-Y plane and 20 nm in the Z axis.

- Dr. Jung-Chi Liao at the Institute of Atomic and Molecular Sciences, Academia Sinica, is an expert in super-resolution imaging. We will collaborate with his group to perform STORM.

Aim 1-2: To examine whether S726 phosphorylation is required for ADD1 localization in mitotic centrosomes.

FLAG epitope tagged-ADD1 (FLAG-ADD1) including WT, S726A and S726D will be transiently expressed in HeLa cells and their localization at centrosomes, in particular in PCM, will be analyzed by confocal microscopy or apotome microscopy.

(1) If the S726A mutant fails to localize to mitotic centrosomes, it suggests that S726 phosphorylation is required for ADD1 localization to centrosomes.

(2) If the S726A mutant is able to localize to mitotic centrosomes, it suggests that ADD1 is first recruited to centrosomes by some centrosomal proteins and subsequently phosphorylated at S726 by a mitotic kinase in the centrosome.

Aim 1-3: To examine the possible role of Myo10 and PCM proteins in the recruitment of ADD1 to centrosomes.

(1) Myo10 has been shown to localize near and at the spindle poles (Woolner *et al.*, 2008). Because ADD1 interacts with Myo10 (Chan *et al.*, 2014), it is possible that Myo10 may recruit ADD1 to the spindle pole. Two different human Myo10 shRNA clones from siRNA core, Academia Sinica, will be used to knockdown Myo10 in HeLa cells. The spindle pole localization of ADD1 in Myo10-depleted HeLa cells will be analyzed by confocal microscopy or apotome microscopy.

(2) The results of Aim 1-1 will shed light on intimate partners of ADD1 in PCM. The PCM proteins that co-localize with ADD1 will be depleted and their effect on ADD1 localization will be analyzed.

Aim 2. To establish the significance of ADD1 phosphorylation at S726 in spindle pole integrity.

Our preliminary results show that the defect of multiple spindle poles caused by ADD1 depletion is the result of centriole splitting rather than centrosome overduplication, cytokinesis failure, or PCM fragmentation (Fig. 5-8). Our data also suggest that phosphorylation of ADD1 at S726 may be important for spindle pole integrity in mitosis. To demonstrate this, the ability of phospho-mimetic mutants S726D and S726E to rescue the defects caused by ADD1 depletion will be examined. Moreover, in addition to S726, S12, S355, S358 and S465 of ADD1 are also phosphorylated during mitosis (Fig. 1A). The significance of these mitotic phosphorylation sites of ADD1 in spindle assembly and spindle pole integrity will be examined.

Aim 2-1: To examine the effect of S726D and S726E mutants on the rescue of centriole splitting caused by ADD1 depletion.

Our preliminary results show that depletion of ADD1 induced centriole splitting and led to multiple spindle poles in mitosis, which was rescued by re-expression of FLAG-ADD1, but not the S726A mutant (Fig. 6). To further establish the significance of ADD1 S726 phosphorylation in spindle pole integrity, FLAG-ADD1 S726D and S726E mutants will be constructed and expressed in ADD1-depleted HeLa cells. The percentage of mitotic cells with multipolar spindle poles and the number of centrioles in each spindle pole will be measured from at least 300 mitotic cells.

Aim 2-2: To examine the significance of the phosphorylation at S12, S355, S358 and S465 in spindle assembly and spindle pole integrity.

Our studies indicate that ADD1 has a dual function on both spindle assembly and spindle pole integrity. The capability of FLAG-ADD1 S12A, S355A, S358A and S465A mutants to rescue the mitotic defects caused by ADD1 depletion, including the spindle detects (spindle elongation and distortion) and the spindle pole defect (centriole splitting), will be examined, as described in the Preliminary Results Fig. 5.

Aim 2-3: To examine the effect of ADD1 depletion on the PCM structure.

(1) The PCM proteins are assembled into a cartwheel-like structure in the centrosome (Lawo *et al.*, 2012). It will be of interest to examine if ADD1 depletion affects such a hierarchically organized structure. The PCM structure in the ADD1-depleted HeLa cells will be visualized by SIM super-resolution microscopy with antibodies specific to the PCM proteins, as described by Lawo *et al.*, 2012.

(2) ADD1 depletion causes centriole splitting, leading to one centriole in the spindle pole. The image of "one centriole in one spindle pole" will be obtained by transmission electron microscopy.

For quantitative studies

1. Design - Is it a questionnaire study or a laboratory experiment? What kind of design do you choose?
2. Subjects or participants - Who will take part in your study ? What kind of sampling procedure do you use?
3. Instruments - What kind of measuring instruments or questionnaires do you use? Why do you choose them? Are they valid and reliable?
4. Procedure - How do you plan to carry out your study? What activities are involved? How long does it take?

About you

- Ask yourself why you are the best person to undertake this project.
- You may wish to provide a small section/paragraph to present how your research interests, previous achievements, relevant professional experience and qualifications will support the completion of your research project.

Conditions of the Research Environment

You could also point out how your project fits with the research environment of your prospective institution and why this institution is the best place to conduct your research, in particular if this will provide you with access to unique expertise, pieces of equipment or data.

Style

A good research project may run the risk of rejection simply because the proposal is poorly written. Therefore, it pays if your writing is coherent, clear and compelling.

- Be clear, objective, succinct and realistic in your objectives
- Use section headings
- Present the information in short paragraphs rather than a solid block of text
- Write short sentences
- If allowed, provide images/charts/diagrams to help break up the text

Technical Tips

- Construct a working hypothesis
- Write a short and concise abstract.
- Construct sub-title and headings.
- Check the flow of the theme after finishing all headings and sub-headings.
- Write main sentence of each paragraph.
- Add flesh to each paragraph.
- Highlight the important points and questions.

Common Mistakes in Proposal Writing

1. Failure to provide the proper context to frame the research question.
2. Failure to delimit the boundary conditions for your research.
3. Failure to cite landmark studies.
4. Failure to stay focused on the research question.
5. Failure to develop a coherent and persuasive argument for the proposed research.
6. Too much detail on minor issues, but not enough detail on major issues.
7. Too much rambling -- going "all over the map" without a clear sense of direction. (The best proposals move forward with ease and grace like a seamless river.)
8. Too many citation lapses and incorrect references.
9. Too long or too short.
10. Slopping writing.

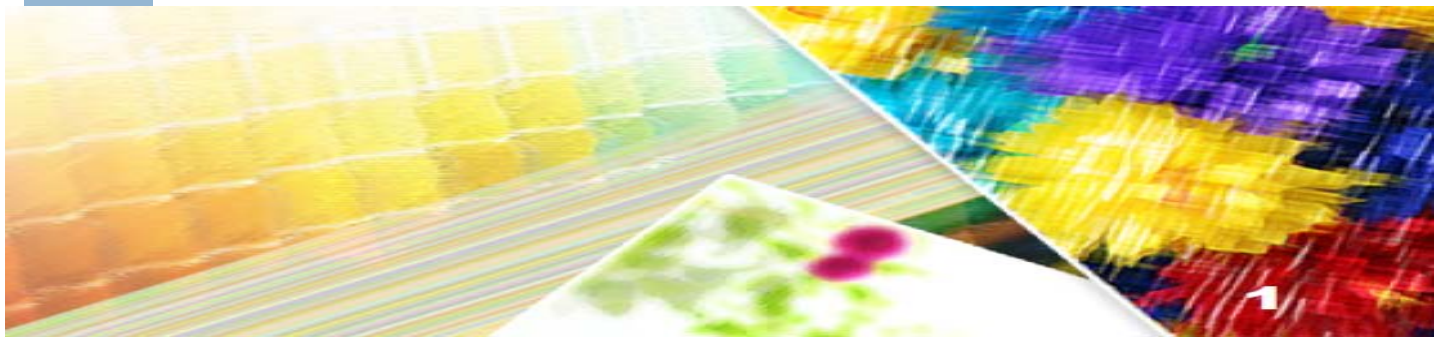
Reviewer's concerns

I. Nature of the Question

II. Approach to the Question

III. Competence of the Investigators

IV. Conditions of the Research Environment



Common rejection reasons

The National Institute of Health (NIH) analyzed the reasons why over 700 research proposal applications were denied. Their findings as to the cause of rejection are worth reviewing:

I. Nature of the research question (18%)

II. Approach to the question (38.9%)

III. Competence of the Investigators (38.2%)

IV. Conditions of the Research Environment (4.8%)

I. Nature of the research question (18%)

A. It is doubtful that new or useful information will result from the project (14%).

B. The basic hypothesis is unsound (3.5%).

C. The proposed research is scientifically premature due to the present inadequacy of supporting knowledge (0.6%).

II. Approach to the question (38.9%)

A. The research plan is nebulous, diffuse and not presented in concrete detail (8.6%).

B. The planned research is not adequately controlled (3.7%).

C. Greater care in planning is needed (25.2%).

   1. The research plan has not been carefully designed (11.8%).

   2. The proposed methods will not yield accurate results (8.8%).

   3. The procedures to be used should be spelled out in more detail (4.6%).

D. A more thorough statistical treatment is needed (0.7%).

E. The proposed tests require more individual subjects than the number given (0.7%).

III. Competence of the Investigators (38.2%)

A. The applicants need to acquire greater familiarity with the pertinent literature (7.2%).
B. The problems to be investigated are more complex than the applicants realize (10.5%).
C. The applicants propose to enter an area of research for which they are not adequately trained (12.8%).
D. The principal investigator intends to give actual responsibility for the direction of a complex project to an inexperienced co-investigator (0.9%).
E. The reviewers do not have sufficient confidence in the applicants to approve the present application, largely based on the past efforts of the applicants (6.8%).

IV. Conditions of the Research Environment (4.8%)

A. The investigators will be required to devote too much time to teaching or other non-research duties (0.9%).
B. Better liaison is needed with colleagues in collateral disciplines (0.4%).
C. Requested expansion on continuation of a currently supported research project would result in failure to achieve the main goal of the work (3.5%).

違反研究倫理之行為，係指具有以下情形者：

1. 造假：虛構不存在之申請資料、研究資料或研究成果。
2. 變造：不實變更申請資料、研究資料或研究成果。
3. 抄襲：援用他人之申請資料、研究資料或研究成果未註明出處。註明出處不當情節重大者，以抄襲論。
4. 隱匿其部分內容為已發表之成果或著作。
5. 未經註明而重複發表，致研究成果重複計算。
6. 研究計畫或論文大幅引用自己已發表之著作，未適當引註。
7. 以違法或不當手段影響論文審查。
8. 論文作者及出處登載不實。
9. 未依照實驗動物保護法之相關規範進行動物實驗。
10. 未依照行政院衛生署醫療法及本院人體試驗標準作業程序等人體實驗相關法規進行人體試驗。
11. 違反智慧財產權。
12. 提供傳媒不當之研究訊息。
13. 將已被接受刊登之研究成果寫成研究計畫提出申請。
14. 其他研究過程之越權或不當行為


Thank
you !