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HOW TO WRITE A RESEARCH GRANT

WRITING A GOOD RESEARCH GRANT

- Creative thinking
- Good writing skill

科技部計畫

- 科技部計畫的通過率對你是沒有意義的
- 問自己的計畫被通過的機會有多少
- 要想辦法讓計畫通過的機會增加

計畫是寫給審查者看的

- 審查者經常是主觀的。
- 審查者是沒有耐心的。
- 審查者不可能完全懂你的計畫。
- 一旦審查者誤解了你的意思，你是沒有機會解釋的。
- 要讓審查者懂你寫的東西。
- 永遠要問審查者會不會不懂這句話。
- 不要得罪人

GET ALL THE HELP THAT YOU CAN GET

- Discuss your ideas with friends
- Use Facebook
- Get critiques from your colleagues

WRITING STYLE

- Simple
- Concise
- Do not create a “maze”; let reviewers clearly understand what you are saying
- Do not repeatedly saying the same things in a grant

TWO BASIC RULES IN WRITING

Loops and hooks
Inverted pyramids

大

青蛙是脊椎動物,牠在外表有一層綠色
的皮膚。綠色的原因是因為在皮膚細
胞之中含有葉綠体。所以青蛙可以利 中
用葉綠体進行光合作用，這是為什麼
青蛙要曬太陽的原因。

小

MOST GRANTS

- 初審兩人
- 複審兩人
- 四個分數平均

群體計畫

- 提出研究大綱
- 初審
- 撰寫計畫
- 複審

EVALUATION

一、**專題研究計畫**：請綜合下列五點審查項目勾選等級及評給分數 (70分)

○ 極優(70-63) ○ 優(62-57) ○ 可(56-50) ○ 差(< 50)　　評分：

1.研究主題之**重要性與創新性**。
2.研究計畫撰寫之完整性及妥適性，**實驗設計及重要研究方法之可行性**。
3.預期成果在學術上或實用上之價值。
4.主持人研究能力及經驗，文獻蒐集之完備性及對國內外相關研究現況是否清楚瞭解。
5.研究人力配置、儀器、經費之申請額度及執行期限之合理性。

二、 **主持人近五年內之研究成果及所反映之學術研究能力**： 請綜合下列二點審查項目勾選等級及評給分數 (30分)

○ 極優(30-27) ○ 優(26-23) ○ 可(22-20) ○ 差(< 20)　　評分：

1. 最近一件執行科技部研究計畫之研究報告及成果是否良好。
2. 近五年發表之研究成果（論文、專利及技轉等）之質與量，在同研究領域同儕中之相對表現。

※等級參考分數：(A)優先推薦(≧ 90) (B)推薦(89- 80) (C)勉予推薦(79-70) (D)不推薦(<70)

總分(上兩項評分相加)：

三、**本計畫是否涉及國家機密或敏感科技?**　　○ 是 ○ 否

四、**本研究計畫若涉及下列實驗，須附相關核准或同意進行實驗之文件**：

EVALUATION

六、綜合審查意見：(本綜合意見為複審及計畫主持人的重要參考資料，敬請務必填寫。請對申請計畫優劣做具且客觀之評述及提供建設性意見與建議，避免使用不當的尖銳文字。請特別留意審查意見及審查評分之優劣應致，勿造成評語佳而評分低之相互矛盾情形。)

1、本計畫研究內容簡述：

2、審查意見：（請分別就前頁之專題計畫及研究成果等項目審查，針對以下四點列舉具體的審查意見及建議字數總和至少200字以上為原則，篇幅以4頁為上限。）

(1) Significance & Novelty：

(2) Weakness：

EVALUATION

(3) PI Performance :

(4) Specific Comment :

CONTENTS

- Abstracts
- Background information
- Research progress and current studies
- Hypothesis
- Significance
- Research design
- Anticipated results

ORDER OF WRITING

- Hypothesis
- Specific aims
- Research design/Research progress and current studies
- Anticipated results
- Background information
- Significance
- Abstracts

給自己足夠的時間撰寫

- 3月：建立hypothesis
- 3-10月：進行支持hypothesis的實驗
- 10月初成立FaceBook群組，開始撰寫
- 11月中初稿完成
- 11月底撰寫完成
- 12月初準備輔助資料
- 12月20日送出

HYPOTHESIS

- has novelty
- is important
- has elements of surprises

PREVIOUS AND CURRENT STUDIES中不可以寫什麼

- 不是要告訴人家你去年發表過多少文章
- 不是要告訴人家你目前實驗室在做什麼
- 和hypothesis無關的結果

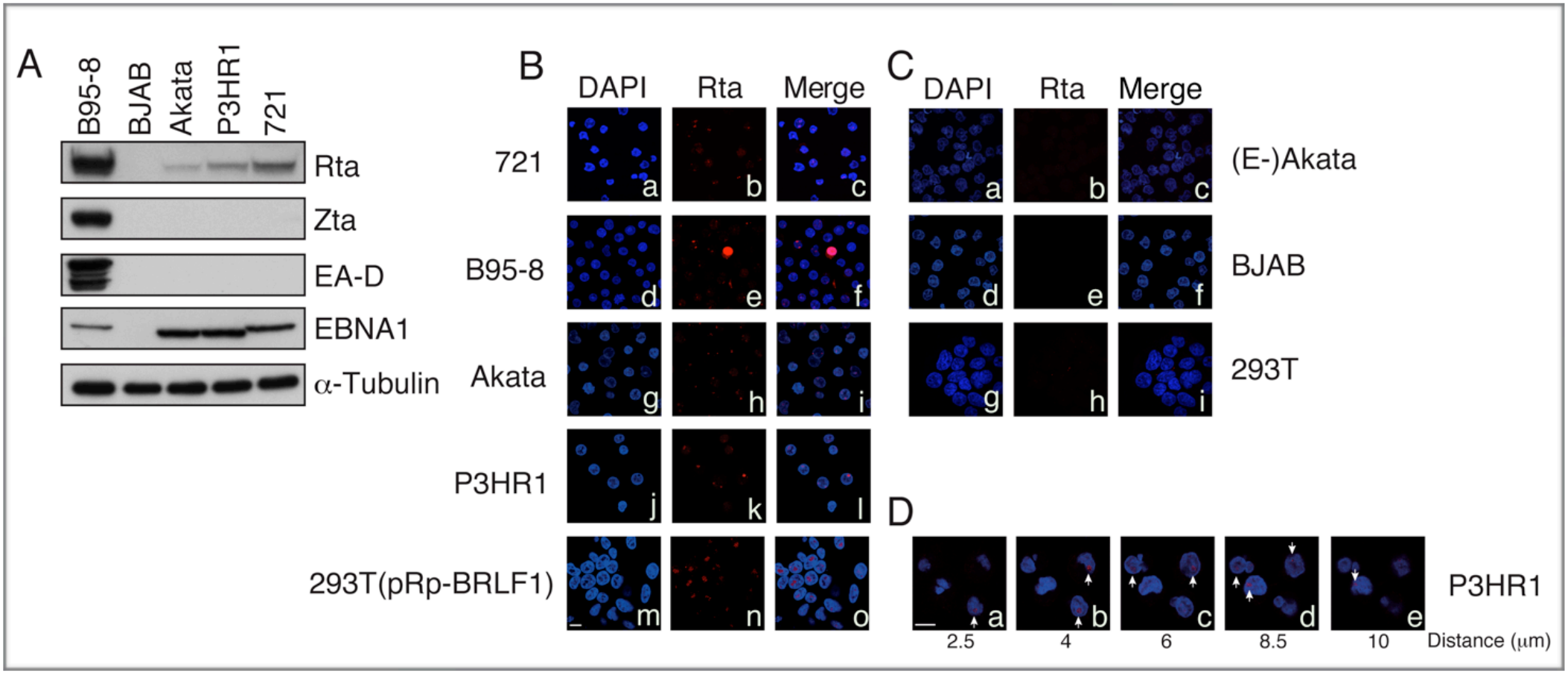
要說服審查者

- 在Previous and Current Studies中提出說服人的證據

寫法

- 只寫能支持你的假說的結果
- 以撰寫結果的方式寫
- 標題要有動詞(下結論)
- 除了在敘述科學事實以外，一律用過去式
- 不要寫過多的結果
- 永遠要問審查者可能會想什麼

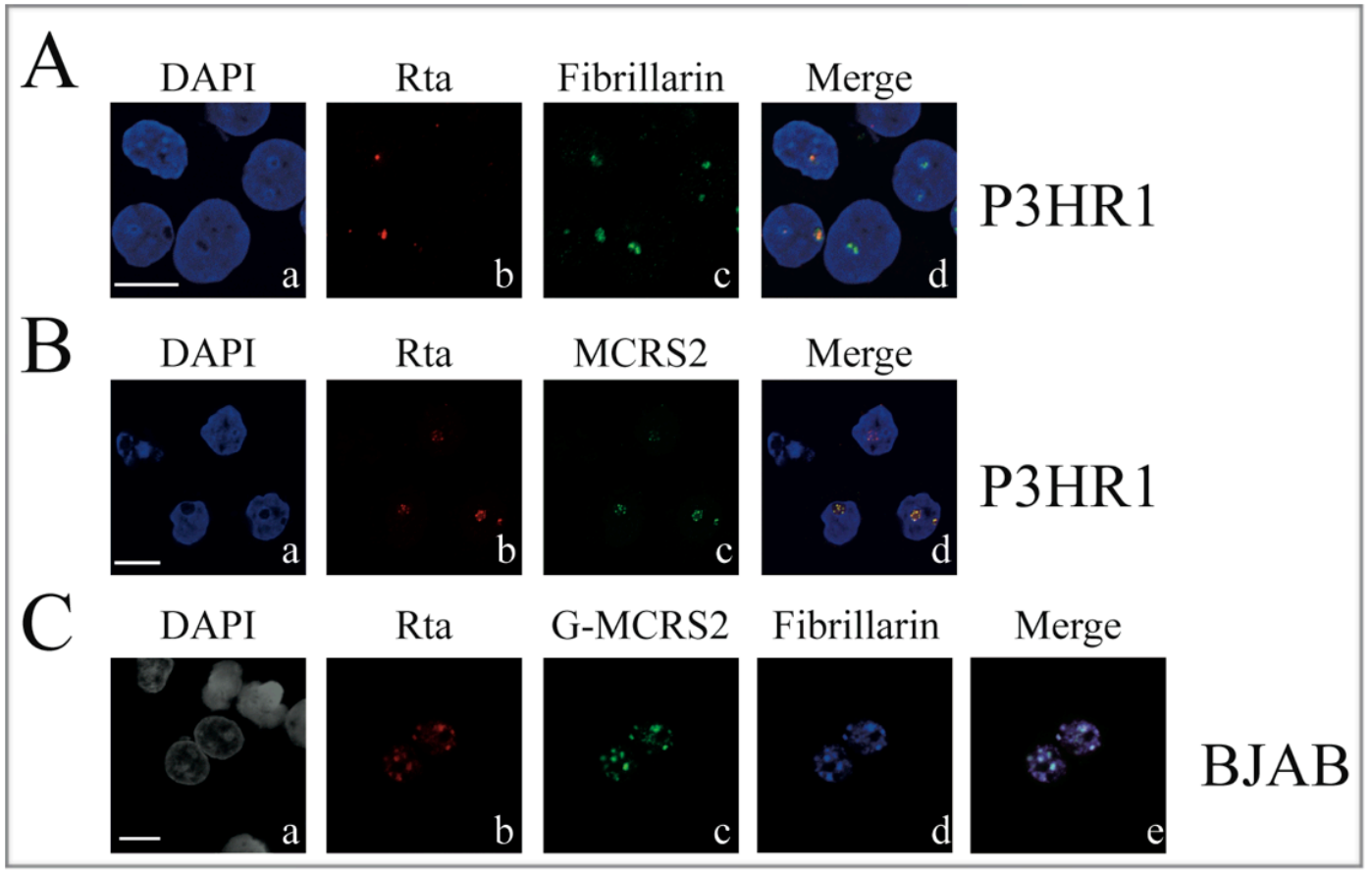
DEVELOPMENT OF A MODEL: RTA IS EXPRESSED DURING EBV LATENCY.



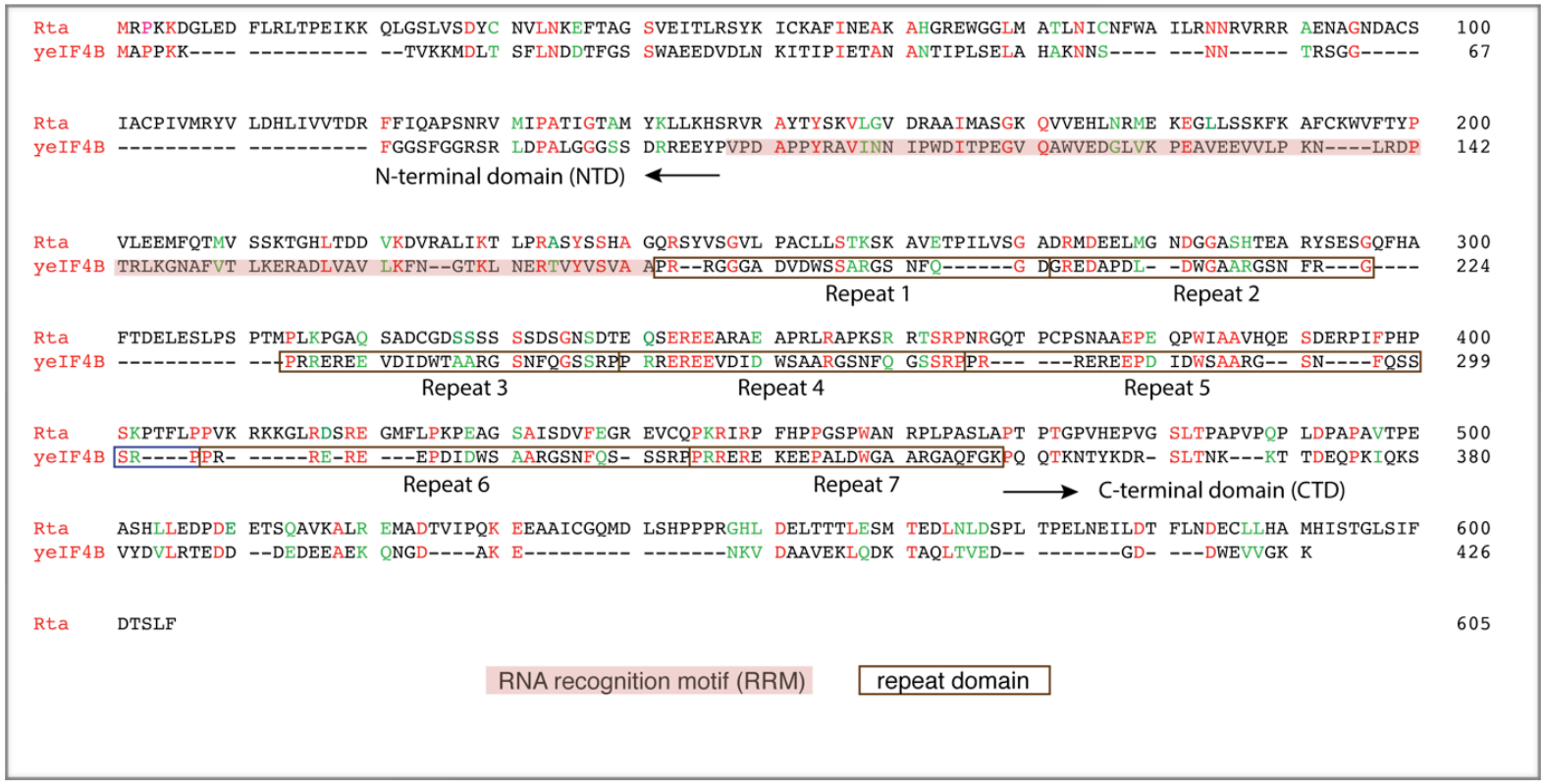
Rta is expressed by cells that are latently infected by EBV.

Rta is a transcription factor, which activates the transcription of many EBV lytic genes (). The transcription of the Rta gene in human B lymphocyte cells is also thought to be repressed, but is strongly activated by Zta and itself after the virus enters the lytic cycle (). However, in immunoblot analyses that were conducted in our laboratories, we irreproducibly detected the expression of Rta in P3HR1 and Akata cells that were not activated for viral lytic replication (data not shown). Since EBV in B lymphocyte cells, B95-8 in particular, is known to enter the lytic cycle spontaneously (), the detection was assumed to be due to the cells had encountered unknown stimuli that triggered the lytic cycle during culturing. However, recently we found that the detection of Rta by immunoblotting from P3HR1 cells, which had not been subjected to EBV lytic activation, improved substantially and the results of immunoblotting became reproducible if a sonication step was implemented after a cell lysate was prepared. By doing so, we were able to detect Rta routinely in the lysates from 721, Akata, B95-8, and P3HR1 cells that were latently infected by EBV (Fig. 1A), showing that Rta is expressed during EBV latency.

DEVELOPMENT OF A MODEL: RTA IS PRESENT IN THE NUCLEOLUS.



DEVELOPMENT OF A MODEL: RTA IS HOMOLOGOUS TO EIF4B.



RESULTS PRESENTED IN PREVIOUS AND CURRENT STUDIES

- More than 95% of EBV-infected B lymphocytes contains Rta speckles.
- Spontaneous lytic activation of EBV in Burkitt's lymphoma cells is less than 0.024%.
- Rta is sequestered in the nucleolus during EBV latency.
- MCRS2 facilitates Rta's nucleolar sequestration.
- Rta loses its ability to activate polII-driven transcription after nucleolar sequestration.
- Rta does not promote polI-driven transcription after nucleolar sequestration.
- Rta is present in the 40S ribosomes.
- Rta enhances translation *in vitro* and *in vivo*

要有另人訝異的因素

- Rta is expressed during the lytic cycle.
- Rta is a transcription factor.
- Rta is an EBV latent protein.
- Rta is a translational initiation factor.

HYPOTHESIS必須有說服力

- 假說經常和科學事實距離八丈遠
- 但與胡扯只有一線之隔
- 說服人家你沒有在胡扯

寫法

- Try to make it concise
- Write a brief introduction
- Try to say only your model
- Use present and future tense

HYPOTHESIS

Our current study shows that Rta is expressed and sequestered in the nucleolus during viral latency. We also find that expression of Rta activates both cap-dependent and IRES-dependent translation. Other important findings made by this study include that Rta binds to m^7GTP-agarose beads (Fig. 12), and after centrifugation through a sucrose gradient, Rta is present in 40S ribosome fractions (Fig. 11), suggesting that Rta participates in translation initiation. Rta although is critically involved in transcription and signal transduction (1, 22, 46), the findings on promoting translation is unexpected. Based on these facts, we propose following models (Fig. 13) to explain how EBV produces Rta during viral latency to affect cellular vitality to relieve the burden of viral infection.

USE PRESENT TENSE

HYPOTHESIS

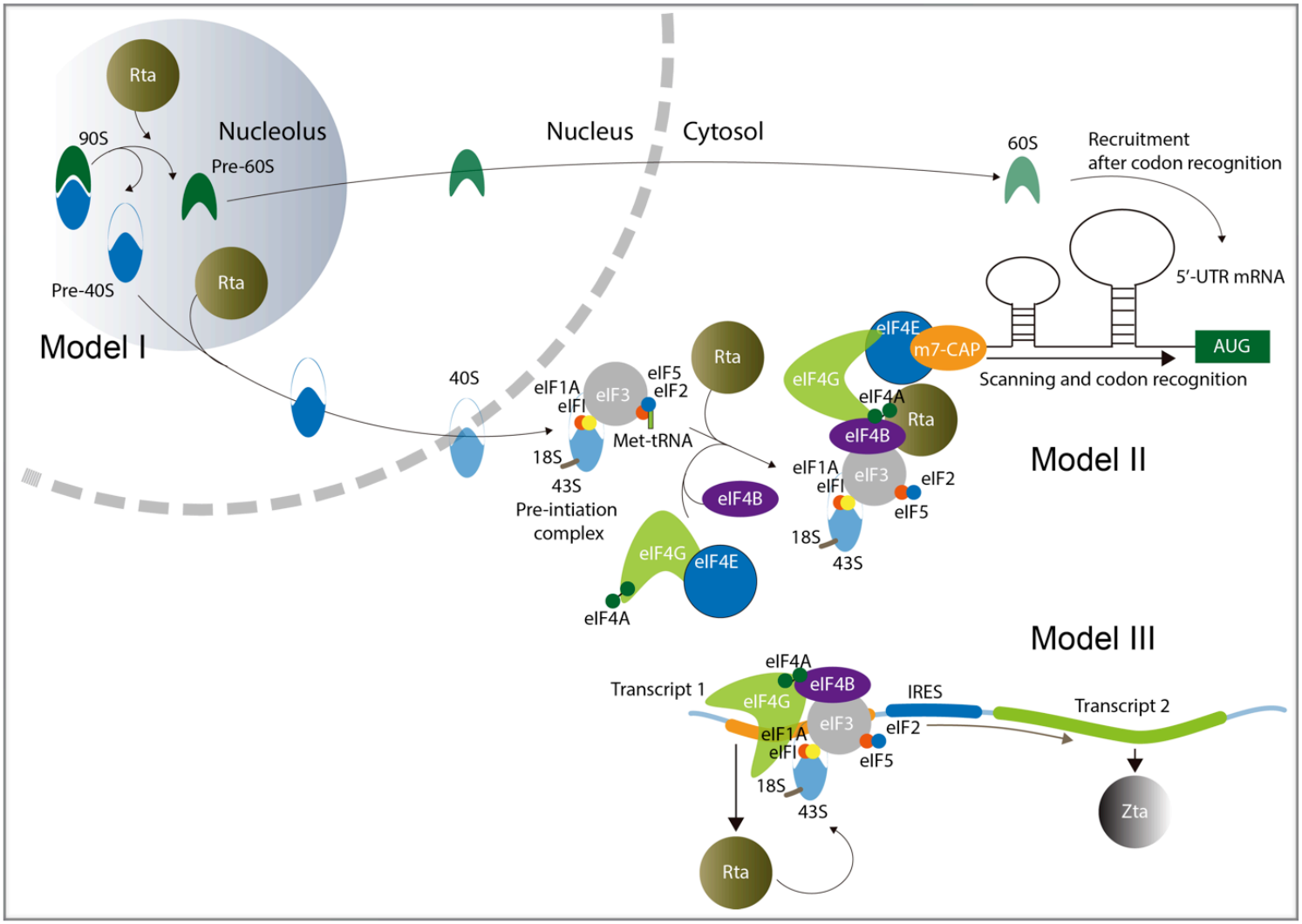
Model 1: Since the nucleolus is the site of ribosome biogenesis, we propose that Rta is incorporated into the 90S pre-ribosome and ultimately becomes a part of PIC. Rta may interact with other translation initiation factors to enhance the binding of PIC to the cap to promote translation initiation. This model also posits that cytoplasmic Rta, rather than the nucleolar Rta, binds to mature 40S ribosome to facilitate cap-dependent translation.

Model 2: Rta participates in translation initiation and substitutes or enhance the functions of eIF4B to promote the recruitment of PIC and its binding to mRNA.

Model 3: In cap-independent translation, Rta functions as an IRES *trans*-acting factor (ITAF) to promote the binding of 43S PIC to IRES to enhance IRES-dependent EV71 translation. In the case of translation of BZLF1 ORF from the bicistronic RZ-mRNA, Rta associates with helix 36 in 18S rRNA in the 40S ribosome after translating BRLF1 ORF. The ribosome then lands on region I in the intercistronic region, and Rta promotes the binding of the 40S ribosome to region I. Initiation factors that are already present at region I or recruited to the region by Rta then associate with PIC to facilitate the scanning of the intercistronic region to start translation at the AUG codon of BZLF1 ORF.

雖然你的理論不見得正確，你要做到讓審查者無法反駁你

ILLUSTRATE YOUR MODEL



IS "SIGNIFICANCE" THAT IMPORTANT IN A GRANT?

- Help your reviewers to write their comments.
- Write 1-2 sentences to describe your system.
- Describe the problems that you want to solve.
- Describe why solving the problems is important.
- Describe what you research will benefit.
- Use present or future tense.

The expression of Rta during viral latency and its involvement in translation initiation are both unexpected and surprising. Results from our study strongly suggest that Rta is expressed during latency, which provides the vigor to the host by increasing translational activities to relieve the burden that is created by viral infection and by the possible energy drain due to the presence of tens of EBV DNA copies in the cell. This is opposite to the facts that many viruses cleave translation initiation factors to prevent cellular translation so that the viruses gain the resources to promote their own protein synthesis (23, 58, 70). Since Rta is expressed constitutively, Rta expression after viral infection may influence EBV gene expression at both transcriptional and translation levels. This proposed study will pave a way leading to the understanding the functions of Rta that have not been explored in the past and how EBV influences its host to benefit its own survival.

WHAT ARE YOUR AIMS?

- Use aims to test your hypothesis
- Aims are the scaffold of your research grant
- The scope of each aim should be broad and meaningful
- Each aim should have a title lacking a verb
- Need two to three sentences to describe each aim
- Let people understand clearly what you will do
- Use present or future tense

This study proposes that Rta is expressed during EBV latency and promotes translation. After synthesis, Rta is sequestered in the nucleolus, where Rta is incorporated into pre-ribosomes. As ribosome biogenesis proceeds, Rta ultimately becomes a part of the 40S ribosome and promotes translation initiation. The aims proposed below will test this hypothesis.

1. Investigating how Rta is incorporated into the ribosome

Since the nucleolus is the place where ribosome biogenesis starts and Rta is present at this subnuclear location, this study will follow the ribosome biogenesis process to elucidate how Rta is incorporated into the ribosome.

2. Elucidating the mechanism by which Rta activates cap-dependent translation

This study reveals that Rta binds to m^7GTP-agarose beads, suggesting that Rta binds the 5' cap of mRNA. Therefore, we will investigate how Rta interacts with translation initiation factors and in what capacity to promote translation initiation.

3. Studying the involvement of Rta in internal translation initiation

Rta is known to promote translation of the downstream open reading frame form the BRLF1-BZLF1 bicistronic mRNA. Our current study also shows that Rta enhances translation initiation from the EV71 IRES. This study will investigate how Rta interacts with the 40S ribosome and initiation factors to allow translation to initiate from an internal initiation site.

不是在寫材料方法

- 寫材料方法是大忌

WRITING MATERIALS AND METHODS SHOULD BE AVOIDED

Experiment designs and the method

A. Induction of xxxx (an established animal model recently developed in our laboratory)

We have recently developed a simple and inexpensive method to induce xxxx. This technique has been successfully used to produce yyy in isolated perfused rat's lungs. Corn oil is mixed with distilled water to form fatty micelles. The micelles will be added into the venous reservoir. Pilot tests are carried out to determine the optimal corn oil concentration and the dose-response relationship. Corn oil of various volume (0.1, 0.2, 0.4, and 0.6 ml) is mixed with distilled water (0.2 ml). In a total of 24 isolated lungs (n=6 for each volume of corn oil), addition of fatty micelles with corn oil caused increases in LW/BW ratio and LWG. Values for the LW/BW ratio (×100) are 1.1±0.4, 2.1±0.8, 2.6±0.6, and 3.1±0.9 for corn oil volumes of 0.1, 0.2, 0.4, and 0.6 ml. The LWG are 0.8±0.3, 1.7±0.5, 2.1±0.6, and 2.5±0.8 g for corn oil volumes of 0.1, 0.2, 0.4, and 0.6 ml. The data indicate that 0.2 ml of corn oil produced greater effect than 0.1 ml ($p < .05$), and >0.2 ml of corn oil did not further increase LW/BW ratio and LWG ($p > .05$). Accordingly, 0.2 ml of corn oil with the same volume of distilled water is the optimal volume mixture to produce ALI. In this study, we will use oleic Acid and/or PMA (Phorbol Myristate Acetate) to induce.

CHIP ASSAY

Cells will be cultured in 150-nm dishes to about 80% confluency before formaldehyde crosslinking, harvesting and chromatin immunoprecipitation (chIP) using standard chip protocols. The mouse polyclonal anti-Rta and anti-Zta antibody will be used in the IP. Dilution buffer and rabbit immunoglobulin will be used as negative controls, The resulting DNA will be analyzed by PCR with primers flanking the ZREs in the BMRF1 promoter. For chIP western analysis, urea-SDS lysis buffer will be used to wash the beads used for IP of DNA protein complexes. The beads will be boiled at 95°C for 5 min. After centrifugation at 4000 rpm for 5 min, the supernatant will be separated by polyacrylamide gel electrophoresis, transferred and blotted with mouse monoclonal antibodies against Zta and Rta.

"METHODS"-- 錯誤的範例

- Cell culture
- Chromatin immunoprecipitation
- siRNA preparation and transfection
- RNA isolation, RT-PCR and northern hybridization
- Immunocytochemical staining
- Immunoblotting
- Animal studies

寫法

- 把Specific Aims的標題列出(不要改任何字)
- 在每一個Aim之下，列出所有要做的實驗
- 在敘述實驗時，先寫實驗的目的。然後敘述你要怎麼做(不是材料方法)。敘述控制組，並且推測結果。告訴人家為什麼預期某個結果。結果不如預期時，代表是什麼意思。
- 在每個Aim的標題之下寫整個Aim的摘要
- 寫一個綜合所有Aims的摘要，放在最前面
- 用現在式及未來式

Research Design

A summary for A, B, C

A. Investigating how Rta is incorporated into the ribosomes

A summary for A

1. The experimental system
2. Demonstrating that Rta is associated with the 40S ribosome
3. Incorporation of Rta into the pre-ribosome
4. Incorporation of Rta into the ribosomes *in vitro*

B. Elucidating the mechanism by which Rta activates cap-dependent translation

A summary for B

1. Studying the interaction of Rta with translation initiation factors
2. Elucidating the initiation factors that interact with Rta
3. Translation initiation by Rta-containing PIC (Rta-PIC)
4. Recruitment of mRNA to 40S ribosome by Rta
5. Binding of Rta to 18S rRNA

C. Studying the involvement of Rta in internal translation initiation

A summary for C

1. Binding of Rta to region I
2. Binding of PIC to region I
3. Studying the translation initiation factors that bind to region I
4. Studying the influence of Rta on EV71 IRES-dependent translation

A. Investigating how Rta is incorporated into the ribosomes

During viral latency, Rta is sequestered in the nucleolus where ribosome biogenesis starts. It is likely that Rta in this subnuclear location is incorporated into the pre-ribosome and ultimately becomes a part of the 40S ribosome to benefit translation initiation. If this is the case, sequestration of Rta in the nucleolus serves an important purpose during EBV latency.

1. The experimental system

Ribosome biogenesis starts in the nucleolus, where ribosomal proteins and rRNA are assembled into a 90S pre-ribosome (6). The pre-ribosome is then converted to the pre-60S and pre-40S ribosomes in the nucleus and they finally mature in the cytoplasm, resulting in the formation of the 60S and 40S ribosomes, respectively. The ribosome in the nucleus and in the cytoplasm can be analyzed by sucrose density gradient sedimentation using a 10-45% gradient and an SW41Ti rotor. After centrifugation and fractionation of the gradient, the presence of Rta and the ribosomes in fractions will be examined respectively by immunoblotting using anti-Rta antibody and antibody that against S6 protein, which is present in the pre-ribosome and the 40S ribosome. Ribosome profiles will be established spectrophotometrically at OD_{254nm} (14). The experimental result shown in Figure 11, which used whole cell extract, illustrates how the experiment is done. Although the ribosome profile shows that Rta is present in the 40S peak (Fig. 11), the results do not necessarily demonstrate the interaction of Rta with the 40S ribosome since the pre-40S ribosome probably also present in these fractions and the sedimentation methods may not have enough resolution to distinguish the two. Similarly, the 90S pre-ribosome will be difficult to separate from the 80S ribosome. Therefore, cell fractionation and a ribosome purification step will be implemented before the density gradient sedimentation experiment is performed. A ribosome purification method established by of Belin *et al*. (4) will be employed for this purpose.

1. Demonstrating that Rta is associated with the 40S ribosome

The purpose of this study is to elucidate how Rta is incorporated into the ribosome. If Rta participates in translation initiation, Rta likely becomes a component of PIC or interacts with eIF4F, which contains eIF4E, eIF4A, and eIF4G (42), at 5' cap of mRNA. To investigate how Rta becomes a part of PIC, this study will use a whole cell extract prepared from 293xER cells (16), which express Rta constitutively from an Rta gene integrated into the chromosome. After removal of the nucleus by low speed centrifugation, ribosomes in the cytoplasm will be purified according to the method of Belin *et al.* (4). Purified ribosome will be fractionated by sucrose density gradient sedimentation and a ribosomal profile will be established. The presence of S6 protein will be demonstrated by immunoblotting to indicate the fractions that contain the 40S and 80S ribosomes. The lysate will also be examined by immunoblotting to demonstrate the presence of cyclophilin A, a cytoplasm marker (4), but not histone H4, a nuclear marker. The presence of cyclophilin A and the absence of histone H4 is important to this study since the nucleus contains the pre-40S ribosome, which has a sedimentation coefficient close to 40S and may not be separated well from the 40S ribosome by centrifugation. If Rta is cofractionated with 40S ribosome, the result will indicate that Rta is incorporated into the 40S ribosome. If Rta is also present in the fractions containing 80S ribosome, the result will indicate that Rta remains associated with the 40S ribosome during translation elongation, although the results shown in Fig. 11 disfavor this possibility. The presence of Rta in the 60S ribosome is not favored by our hypothesis as the 60S ribosome does not participate in translation initiation (42) and the results shown in Fig. 11 suggest such a fact. An immunoblot study will also reveal whether Rta is phosphorylated, as phosphorylated and unphosphorylated Rta can be distinguished by gel electrophoresis and immunoblotting. Since translation initiation factors are often phosphorylated (29), if only phosphorylated Rta is incorporated into ribosome, the result will suggest that Rta's phosphorylation is important to translation.

如何預期你的結果

- 先寫一小段背景資料去敘述你的系統，並說明你的假說。
- 如果第一個假說是對的，你的實驗設計中會得到什麼結果。
- 如果結果不如預期，那代表是什麼意思。
- 告訴人家不如預期的機會不大，為什麼？
- 告訴人家你可能會遭遇的困難(不要太多)。
- 接著寫第二第三個假說。
- 寫一段結論告訴人家你的研究有多偉大。
- 用現在式及未來式

Rta is known to activate the genes that are important to viral lytic development (55). However, our current study shows that Rta is expressed during viral latency and sequestered in the nucleolus. We also show that the sequestration prevents Rta from activating PolII-driven transcription and does not seem to activate the transcription of the rDNA gene. Rather, the expression of Rta promotes translation. We also show that Rta binds to m^7GTP-agarose beads, suggesting that Rta binds to the 5' cap of mRNA and participates in translation initiation. The finding also suggests that the promotion of both cap-dependent and cap-independent IRES translation by Rta is not caused by a possible enhancement of transcription of the genes that are involved in translation initiation. Furthermore, Rta is required for translation of the downstream BZLF1 ORF from the bicistronic RZ-mRNA. We propose that Rta facilitates the binding of the 40S ribosome to the intercistronic region to initiate translation of BZLF1 ORF (Fig. 13).

ANTICIPATED RESULTS

1. We first propose that Rta is incorporated into the pre-ribosome in the nucleolus during ribosome biogenesis. In this case, the effect of Rta on translation should be a general one and is not limited to the translation from a small subset of mRNA. If this is the case, Rta should be present in the 90S pre-ribosome as well as 40S ribosome fractions in a sucrose density gradient.
2. Based on the facts that the sequence of Rta is similar to that of eIF4B and may bind to 18S rRNA, possibly helix 36 (13), which is adjacent to the binding site for eIF4B, helix 34, of 18S rRNA. We propose that Rta either acts alone or promotes the functions of eIF4B to enhance translation. To this end, we will determine whether Rta promotes the binding of 40S ribosome to mRNA, an important function of eIF4B (51). If Rta indeed has this function, the formation of mRNA-43S ribosome should increase in 293 cells after transfecting the cells with pCMV-Rta. Binding assay using 43S ribosome purified from 293T(pCMV-3) and 293T(pCMV-Rta) cells should also reveal this function.

ANTICIPATED RESULTS

Rta is an amazing protein with diverse functions. Not only does the protein activate transcription of both viral and host genes, but also promotes translation. Rta may have a critical role in relieving the burden of EBV infection. Our research will open a research area, which will be important to the understanding on how Rta affects viral infection, immortalization, and oncogenesis.

如果撰寫背景資料

- 先列出大綱，只寫和計畫有關的資料
- 大綱要尊守Inverted Pyramids的原則
- 每段文章要尊守Loops and Hooks的原則
- 用圖説明
- 注意時態

Epstein-Barr virus

Zta and Rta

MSP58/MCRS1/MCRS2

Ribosome biogenesis and translation initiation

Translation of the BZLF1 open reading frame (ORF) from bicistronic BRLF1-BZLF1 mRNA

如何撰寫摘要

- 要簡潔，愈短愈好
- 要評審者知道計畫的精華
- 一兩句背景資料
- 目前的問題所在
- 想要達成什麼目標
- 列出計畫目標
- 用一句話說你的研究有多偉大
- 用現在式及未來式
- 中英文摘要要一致

ABSTRACT

Rta of Epstein-Bar virus (EBV) is a transcription factor encoded by BRLF1. Since transcription of BRLF1 is strongly activated after the virus enters the lytic cycle and Rta's expression is required for EBV lytic development, Rta is considered to be a viral lytic protein and expressed only during the lytic cycle. Contrary to what is generally believed, this study finds that Rta is expressed during viral latency and sequestered in the nucleolus. When present in the nucleolus, Rta loses it ability to activate transcription that is driven by RNA polymerase II, explaining why Rta does not activate EBV lytic genes during EBV latency. On the other hand, although Rta is present in the nucleolus, where rDNA is transcribed, Rta does not influence the transcription of rDNA. Rather, we find that Rta promotes both cap-dependent and cap-independent translation. Our earlier studies also revealed that Rta has a sequence similar to that of yeast eIF4B and promotes Zta translation from an internal translation initiation site in the intercistronic region of the BRLF1-BZLF1 bicistronic mRNA, suggesting that Rta acts as a translation initiation factor. Therefore, this investigation will test the hypothesis that Rta is incorporated into pre-ribosomes in the nucleolus, where ribosome biogenesis occurs. As ribosomes mature, Rta becomes a part of the 40S ribosome and then promotes the binding of the ribosome to the 5' cap of mRNA to enhance translation. Similar translational activation by Rta is also proposed for translation initiation from EV71 IRES mRNA and BRLF1-BZLF1 bicistronic mRNA. This study will elucidate an important function of Rta and reveal how EBV increases translation efficiency to relieve the burden of its host after infection.

TITLE

- The investigation of the sequestration of Rta of Epstein-Barr virus in the nucleolus and its involvement in translational initiation during viral latency
- Sequestration of Rta of Epstein-Barr virus in the nucleolus and its involvement in translational initiation during viral latency
- Translational regulation by Rta of Epstein-Barr virus

關鍵詞

- 尋找審查者的依據