How to Read the Scientific Literature

Jessica Harrell, Ph.D.

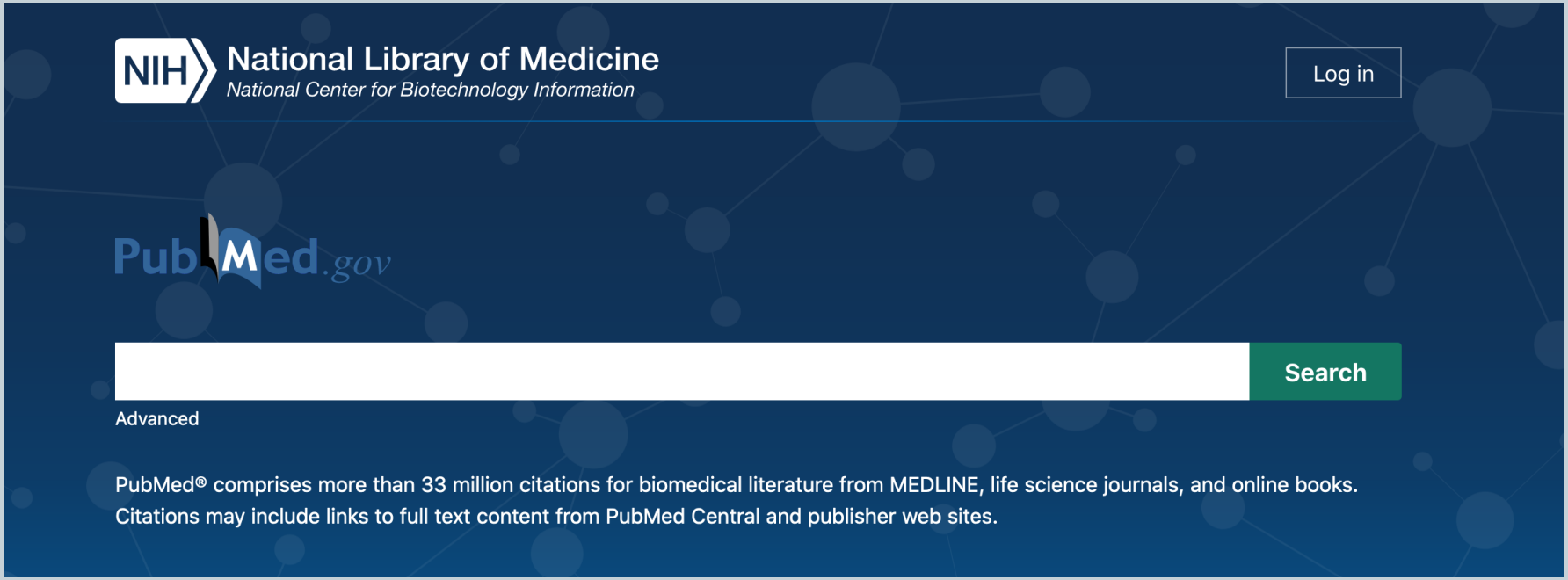
Duke | UNDERGRADUATE RESEARCH SUPPORT OFFICE

Adapted from presentations by Ashalla Freeman, Ph.D. and Kelly Hogan, Ph.D.

What does it mean to critically analyze research literature?

Critical analysis is **the detailed examination and evaluation of another person's ideas or work**.

Where Do You Start?



https://pubmed.ncbi.nlm.nih.gov/?myncbishare=dukemlib&dr=abstract&otool=dukemlib

Primary Articles vs. Review Articles

Article Characteristics

Primary Research Articles

- Original research from experiments and studies
- Include methods section
- Include results section
- Often include hypothesis

Review Articles

- Summarize, analyze, and critique existing research
- Often more general topics
- Often do not have a methods section
- Often have review in the title

Do's and Don'ts of Reading the Scientific Literature

- What are some approaches that you've used, successfully or not so successfully?

How to approach the research literature:

- ***DO NOT*** try to learn a large number of facts!
- ***DO*** determine what is unknown and what the paper aims to address!
- ***DO*** get a sense of what methods and experiments have been done to address particular questions!
- ***DO*** analyze the results yourself!
- ***DO*** examine the controls!
- ***DO*** ask questions!
- ***DO*** look up things you don't know or understand!
- ***DO*** TAKE NOTES!

How is a Paper Organized?

- Title/Authors
- Abstract (summary)
- Introduction
- Materials and Methods
- Results and Figures
- Discussion
- References

Biochemical and Biophysical Research Communications 408 (2011) 489–493

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PARP-1 regulates the expression of caspase-11

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ARTICLE INFO

Article history:
Received 7 April 2011
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Keywords:
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Caspase-11
LPS
NF-κB
Inflammation

ABSTRACT

Poly(ADP-ribose) polymerase-1 (PARP-1) is a multifunctional enzyme that regulates DNA repair, cell death and transcription of inflammatory proteins. In the present study, we present evidence that PARP-1 regulates the expression of caspase-11 following lipopolysaccharide (LPS) stimulation. Knockdown of PARP-1 suppressed the LPS-induced expression of caspase-11 at both mRNA and protein levels as well as caspase-11 promoter activity. Importantly, PARP-1 was recruited to the caspase-11 promoter region containing predicted nuclear factor (NF)-κB-binding sites when examined by chromatin immunoprecipitation assay. However, knockdown of PARP-1 did not suppress the expression of caspase-11 induced by interferon-γ that activates signal transducer and activator of transcription 1 but not NF-κB. PARP-1 enzymatic activity was not required for the caspase-11 upregulation since pharmacological inhibitors of PARP-1 did not suppress the induction of caspase-11. Our results suggest that PARP-1, as a transcriptional cofactor for NF-κB, regulates the induction of caspase-11 at a transcriptional level.

1. Introduction

Caspase-11, a murine caspase of the caspase-1 subfamily of cysteine proteases, plays an important role in the regulation of inflammation and apoptosis [1–3]. The expression of caspase-11 is barely detectable under normal condition but highly inducible by pro inflammatory or cytotoxic stimuli [1,2]. Following induction, caspase-11 can regulate inflammatory responses by activating caspase-1 and thereby producing mature interleukin (IL)-1β or IL-18 [2,3]. In addition, it can directly activate caspase-3 to regulate apoptosis [1]. A critical involvement of caspase-11 has been reported in many disease models accompanying pathological apoptosis and inflammation such as brain ischemia, LPS-induced septic shock, experimental autoimmune encephalomyelitis, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced Parkinson's disease, and spinal cord injury in mice [1,2,4–6]. Therefore, clear understanding of the induction mechanism of caspase-11 is necessary to develop therapeutic strategy targeting caspase-11 induction.

It was reported that proinflammatory stimulators like LPS and interferon-γ (IFN-γ) induce the expression of caspase-11 by activating nuclear factor (NF)-κB and signal transducer and activator of transcription 1 (STAT1), respectively [7]. The signaling pathway leading to caspase-11 induction has been most studied in the LPS-stimulation model. It was shown that LPS-activated toll-like receptor 4 produces reactive oxygen species via interaction with NADPH oxidase complex, which activates mitogen-activated kinase (MAPK) signaling involving apoptosis signal-regulating kinase 1, p38 MAPK or c-Jun N-terminal kinase and ultimately NF-κB to activate caspase-11 promoter [7–10].

Recently, PARP-1 has been shown to function as a transcriptional coactivator of NF-κB and regulates the expression of several proinflammatory genes such as tumor necrosis factor (TNF)-α, IFN-γ and inducible nitric oxide synthase (iNOS) [11–13]. Therefore, it is plausible that caspase-11 whose expression is under the control of NF-κB is also regulated by PARP-1 for its induction. In the present study, we show evidence that the induction of caspase-11 is indeed regulated by PARP-1. Knockdown of PARP-1 suppressed the expression and promoter activity of caspase-11 following LPS stimulation. Moreover, PARP-1 was chromatin-immunoprecipitated on the caspase-11 promoter region containing NF-κB-binding sites. The involvement of PARP-1 in the induction of caspase-11 seemed to be independent of its enzymatic activity and specific for the NF-κB- but not STAT1-activating stimuli. Our results suggest that PARP-1 functions as a coactivator of NF-κB to induce caspase-11 in response to LPS stimulation.

2. Materials and methods

2.1. Mouse embryonic fibroblast cultures

Following removal of head, limbs, and internal organs from 14d gestation mouse embryos, the remaining tissues were minced and

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E-mail address: sjkang@sejong.ac.kr (S.J. Kang).
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Read the Title and Abstract first

Title should be descriptive – often states the main finding

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[1] These authors equally contributed to this work.

The Abstract

- **Carefully read the abstract – it should give a good and thorough idea of what the paper is about**
  - Do you know enough to appreciate this paper?
  - Where will this new knowledge integrate into your previous knowledge?
- **Start to set up your expectations**

The Abstract

- Pick out the General Aim (What is the problem?/Gap in knowledge)
- Pick out the Hypothesis (The solution or answer to the problem).
- Pick out the Specific Aims (Result 1, Result 2….etc)
- Discussion: What are the implications:

The Abstract

General Aim:
What's the problem?

Hypothesis:
Solution or answer to the problem?

Specific Aims:
Test the Hypothesis
- Result 1
- Result 2
- etc, ...

Discussion:
What are the implications?

Pancreatic ductal adenocarcinoma (PDAC) originates from normal pancreatic ducts where digestive juice is regularly produced. It remains unclear how PDAC can escape autodigestion by digestive enzymes. Here we show that human PDAC tumour cells use gasdermin E (GSDME), a pore-forming protein, to mediate digestive resistance. GSDME facilitates the tumour cells to express mucin 1 and mucin 13, which form a barrier to prevent chymotrypsin-mediated destruction. Inoculation of GSDME−/− PDAC cells results in subcutaneous but not orthotopic tumour formation in mice. Inhibition or knockout of mucin 1 or mucin 13 abrogates orthotopic PDAC growth in NOD-SCID mice. Mechanistically, GSDME interacts with and transports YBX1 into the nucleus where YBX1 directly promotes mucin expression. This GSDME–YBX1–mucin axis is also confirmed in patients with PDAC. These findings uncover a unique survival mechanism of PDAC cells in pancreatic microenvironments.

The Abstract

Abstract

Poly(ADP-ribose) polymerase-1 (PARP-1) is a multifunctional enzyme that regulates DNA repair, cell death and transcription of inflammatory proteins. In the present study, we present evidence that PARP-1 regulates the expression of caspase-11 following lipopolysaccharide (LPS) stimulation. Knockdown of PARP-1 suppressed the LPS-induced expression of caspase-11 at both mRNA and protein ieveis as well as caspase-11 promoter activity. Importantly, PARP-1 was recruited to the caspase-11 promoter region containing predicted nuclear factor (NF)-κB-binding sites when examined by chromatin immunoprecipitation assay. However, knockdown of PARP-1 did not suppress the expression of caspase-11 induced by interferon-γ that activates signal transducer and activator of transcription 1 but not NF-κB. PARP-1 enzymatic activity was not required for the caspase-11 upregulation since pharmacological inhibitors of PARP-1 did not suppress the induction of caspase-11. Our results suggest that PARP-1, as a transcriptional cofactor for NF-κB, regulates the induction of caspase-11 at a transcriptional level.

Now flip through the article…

- Note the headings
- Study the figures
- Illustrations
- Tables
- Legends
- Review/Skim the methods – is this a new approach to your research?

- **Get a feel for what's going on…**

How to Approach the Introduction

- **Answer these questions:**
  - What larger question is this research a part of?
  - What is prior research vs. what is being studied here?
  - What data led directly to the work of this paper?
  - What's the hypothesis being tested? How will it be tested?
  - What are the basic conclusions? (Scientists don't really like surprise endings and this is usually stated in the last paragraph of the Introduction.)

Read the Introduction and identify the answers to these questions in groups of 2.

Keywords:
PARP-1
Caspase-11
LPS
NF-κB
Inflammation

as well as caspase-11 promoter activity. Importantly, PARP-1 was recruited to the caspase-11 promoter region containing predicted nuclear factor (NF)-κB-binding sites when examined by chromatin immunoprecipitation assay. However, knockdown of PARP-1 did not suppress the expression of caspase-11 induced by interferon-γ that activates signal transducer and activator of transcription 1 but not NF-κB. PARP-1 enzymatic activity was not required for the caspase-11 upregulation since pharmacological inhibitors of PARP-1 did not suppress the induction of caspase-11. Our results suggest that PARP-1, as a transcriptional cofactor for NF-κB, regulates the induction of caspase-11 at a transcriptional level.

Make notes...

LPS → NF-κB → caspase-11

IFN-γ → STAT1 → caspase-11

1. Introduction

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programmed cell death

Identify the larger question

It was reported that proinflammatory stimulators like LPS and interferon-γ (IFN-γ) induce the expression of caspase-11 by activating nuclear factor (NF)-κB and signal transducer and activator of transcription 1 (STAT1), respectively [7]. The signaling pathway leading to caspase-11 induction has been most studied in the LPS-stimulation model. It was shown that LPS-activated toll-like receptor 4 produces reactive oxygen species via interaction with NADPH oxidase complex, w activates mitogen activated kinase
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The question being addressed....

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How will it be ted?

What previous research has been done?

What data has led to the work of this paper?

nd methods

bryonic fibroblast cultures

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Basic Conclusion

E-mail address: sjkang@sejong.ac.kr (S.J. Kang).
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Materials and Methods

- Should be detailed enough for another scientist to replicate the work (volumes, times, company material was purchased from etc.) –
  Often, this section is compressed and you may need to look up another paper that is referenced for more detail.
- ***And then another…and another….***
  - What was measured? How?
  - Sample number? (Did they do this more than once?)
  - Conditions? (Am I looking at a reduced or non-reduced protein gel?)
- Do you know enough about the method to understand why and how they used it?

Materials and Methods

EDTA (JBI). The dissociated cells were resuspended in Dulbecco's modified Eagle's medium (DMEM; JBI) supplemented with 10% fetal bovine serum (FBS; JBI) and 1% penicillin/streptomycin and anti-mycotics solution (JBI). Mouse embryonic fibroblasts (MEFs) were maintained at 37 °C in the 5% CO_2 incubator. The medium was changed every third day.

2.2. RNA interference of PARP-1 and transfection

Expression of PARP-1 was knocked-down using lentiviral PARP-1 short-hairpin RNA (shRNA) constructs created in pLKO.1 vector system (Open biosystems). Transient expression of each vector (3 μg of DNA/35 mm dish) in MEFs was accomplished with TransIT-LT1 transfection reagent according to the manufacturer's protocol (Mirus). After overnight incubation with the transfection reagents, the medium was changed with fresh growth medium. Among 5 different vectors for different target sequences of PARP-1, two (#1 and #4) were confirmed to have maximal knockdown effect upon immunoblot assay. The hairpin sequence for #1 is CGG GAC GAA CTC CTA TTA CAA ATA GTG AAG CCA CAG ATG TAT TTG TAA TAG GAG TTC GTC CCT and for #4 is CCT CGA AAA ACT TCA CAA AGT ATA GTG AAG CCA CAG ATG TAT ACT TTG TGA AGT TTT TCG AGT. pLKO.1 for control shRNA was also purchased from Open biosystems.

2.3. Immunoblot assay

The attached cells were washed three times with ice-cold phosphate-buffered saline and harvested with lysis buffer (150 mM NaCl, 50 mM Tris–HCl pH 8.0, 1% Nonidet P-40, 0.5% Sodium deoxy-

visualized by ethidium bromide staining.

2.5. Caspase-11 promoter assay

To measure caspase-11 promoter activity, MEFs were transfected with pGL3-caspase-11 promoter and control pRL-TK plasmids and incubated for 36 h. Following drug treatment, dual luciferase activity assay was performed using dual luciferase reporter assay kit (Promega) according to the manufacturer's protocol. pGL3-caspase-11 promoter construct was a generous gift from Dr. R. Beyaert (Ghent University, Belgium) and pRL-TK used as an internal control was purchased from Promega.

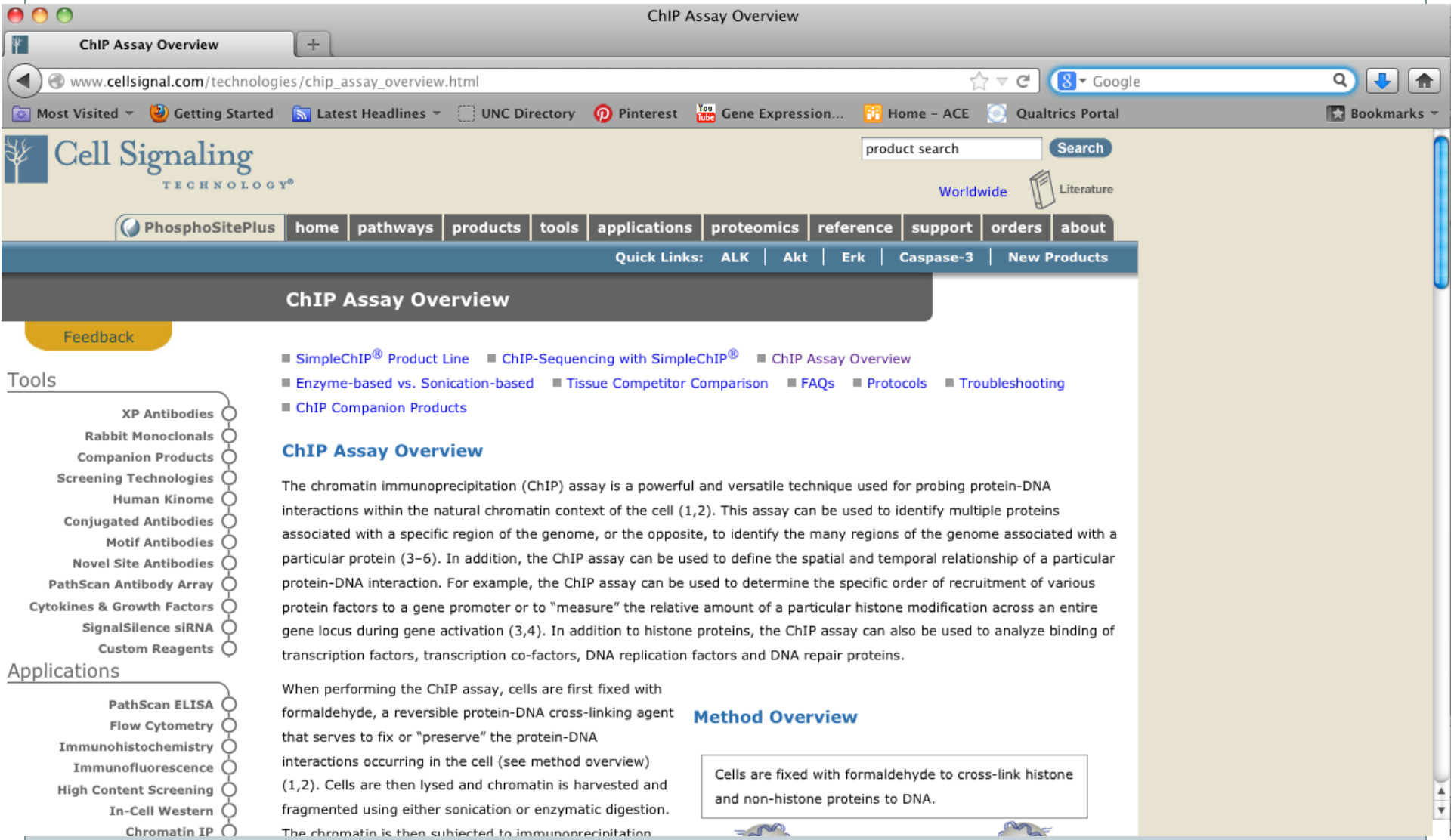
2.6. Chromatin immunoprecipitation assay

Chromatin immunoprecipitation (ChiP) was performed using EZ-ChiP kit (Millipore) according to the manufacturer's protocol. Polyclonal anti-PARP-1 antibody was purchased from Abcam (ab6079). The primer pair for the PCR was chosen from the caspase-11 promoter spanning three reported NF-κB binding sites [7]. Forward; 5′-TGC AGC CGC TTT AGT TGT G-3′ and reverse; 5′-CTC AGT TCT TTC GCA CTG TG-3′ were used.

2.7. Antibodies and reagents

The primary antibodies used in this study are as follows: anti-caspase-11 rat monoclonal antibody (1:200, Abcam), anti-PARP-1 mouse polyclonal antibody (1:500, BD Bioscience), anti-α-tubulin mouse monoclonal antibody (1:4000, Sigma). IFN-γ was purchased from R&D. 3,4-Dihydro-5-[4-(1-piperidinyl)butoxyl]-1(2H)-iso-

Google Your Methods!



ChIP Assay Overview

ChIP Assay Overview

www.cellsignal.com/technologies/chip_assay_overview.html

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- PathScan ELISA
- Flow Cytometry
- Immunohistochemistry
- Immunofluorescence
- High Content Screening
- In-Cell Western
- Chromatin IP
- PTMScan Proteomics

When performing the ChIP assay, cells are first fixed with formaldehyde, a reversible protein-DNA cross-linking agent that serves to fix or "preserve" the protein-DNA interactions occurring in the cell (see method overview) (1,2). Cells are then lysed and chromatin is harvested and fragmented using either sonication or enzymatic digestion. The chromatin is then subjected to immunoprecipitation using antibodies specific to a particular protein or histone modification. Any DNA sequences that are associated with the protein or histone modification of interest will co-precipitate as part of the cross-linked chromatin complex and the relative amount of that DNA sequence will

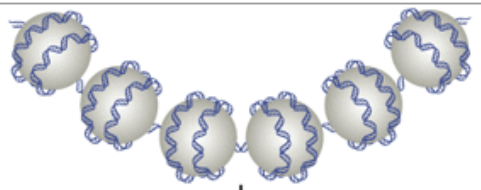
Its difficult to appreciate why they used a particular method unless you understand the basic premise behind the method

:he
ith a

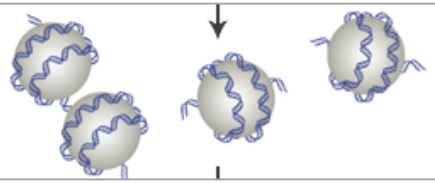
DNA sequence enriched by a protein-specific immunoprecipitation versus an immunoprecipitation with a non-specific antibody control. PCR products are run on an agarose or acrylamide gel to facilitate quantification, and the level of enrichment of the DNA sequence is determined relative to the total amount of input DNA (percent of input). The level of enrichment can also be expressed as fold enrichment above background (enrichment relative to

Method Overview

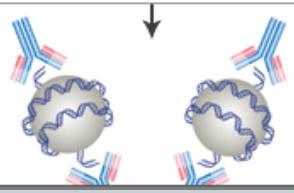
Cells are fixed with formaldehyde to cross-link histone and non-histone proteins to DNA.



Chromatin is digested with Micrococcal Nuclease into 150–900 bp DNA/protein fragments.



Antibodies specific to histone of non-histone proteins are added and the complex co-precipitates and is captured by Protein G Agarose or Protein G magnetic beads.



THE RESULTS SECTION

- Take notes, giving yourself a place to refer to about each figure.
- With each experiment/figure you should be able to explain ***QPRC***:

1) *Q*uestions being answered
2) basic *P*rocedure used
3) the *R*esults
4) the *C*onclusion

Results Section

- Look at the Figure and get an idea of what you think it is showing you
- Now, read the Materials & Methods, Results, and Legend that go along with the Figure – together, these three pieces should explain what was done, how, why, and what was found.
  - …does it make sense to you?
  - …was your idea about what was going on correct?
  - … if not, why not? What don't you understand?

Read Actively!

lowing LPS stimulation.

binding of NF-κB whereas IFN-γ induces STAT1 binding on their

Does PARP-1 regulate expression of caspase-11? What happens to caspase-11 expression when PARP-1 is k/d?

WB-protein levels

k/d PARP stimulate inflammatory response w/ LPS

WB on Casp-11 expression

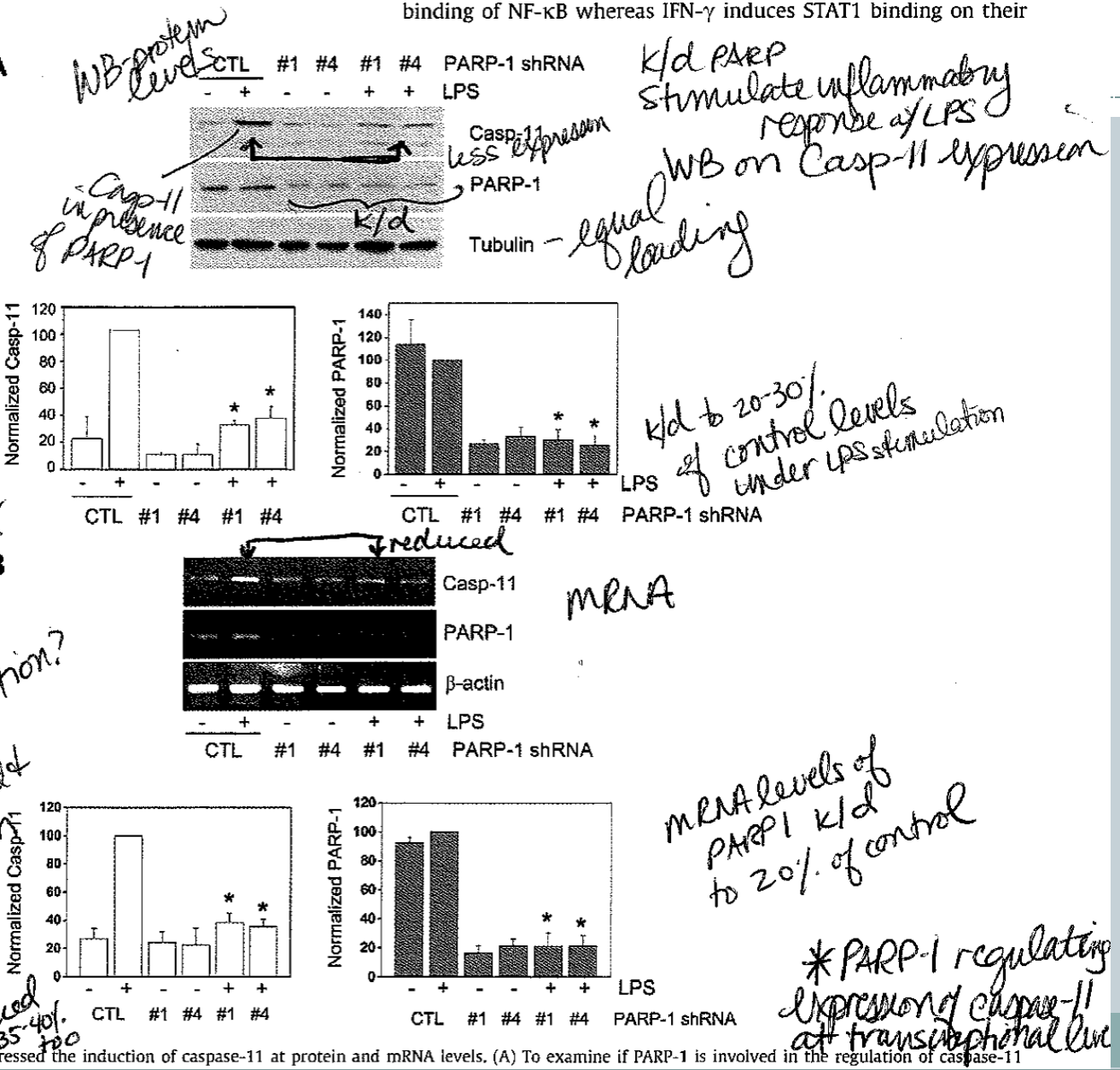


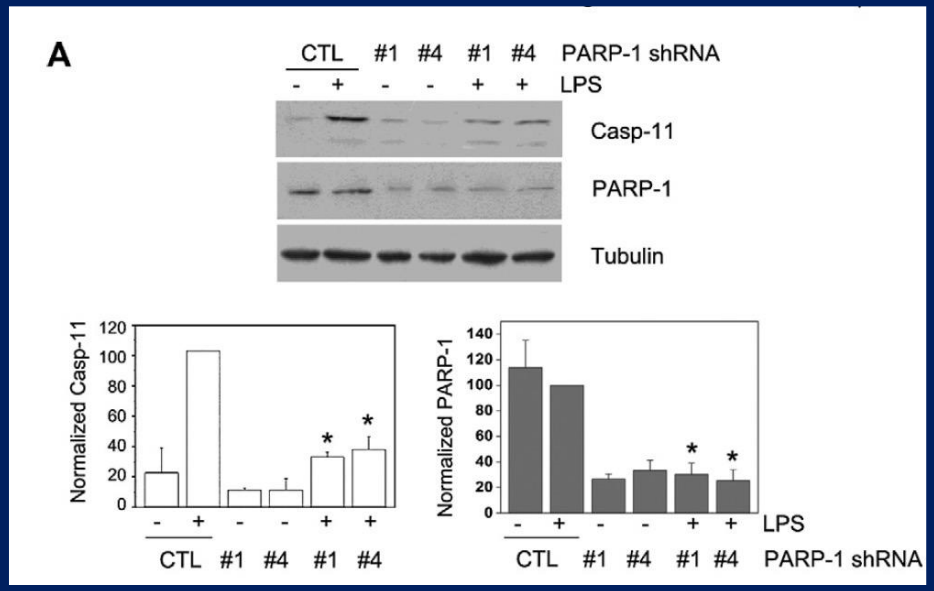
Fig. 1. Knockdown of PARP-1 suppressed the induction of caspase-11 at protein and mRNA levels. (A) To examine if PARP-1 is involved in the regulation of caspase-11

3. Results

3.1. Knockdown of PARP-1 suppressed the induction of caspase-11

It has been reported that PARP-1 regulates the induction of pro-inflammatory genes like iNOS and TNF-α as a transcriptional coactivator of NF-κB [11]. Since induction of caspase-11, a proinflammatory caspase, has been shown to be dependent on NF-κB [7], it is possible that PARP-1 participates in the induction of caspase-11 as well. To test this possibility, we examined the effect of PARP-1 knockdown on the induction level of caspase-11 following LPS stimulation. The MEFs were transfected with control or pLKO.1 vectors containing 21 base-pair hairpin sequences targeting mouse PARP-1. At 48 h after the transfection, the cells were treated with LPS (1 μg/ml) and further incubated for 6 h, followed by immunoblot assay for the detection of caspase-11 and PARP-1. As shown in Fig. 1A, when PARP-1 expression was reduced by RNA interference, the level of caspase-11 protein was reduced by 60% compared to that of control pLKO.1-transfected cells following LPS stimulation. This result suggests that PARP-1 is required for the LPS-induced expression of caspase-11.

To determine whether PARP-1 is required for the transcriptional activation of caspase-11 gene, we examined the mRNA level of caspase-11 in PARP-1 knocked-down MEFs by RT-PCR at 2 h after LPS



Q: Does PARP-1 participate in the induction of caspase-11 following LPS stimuation?

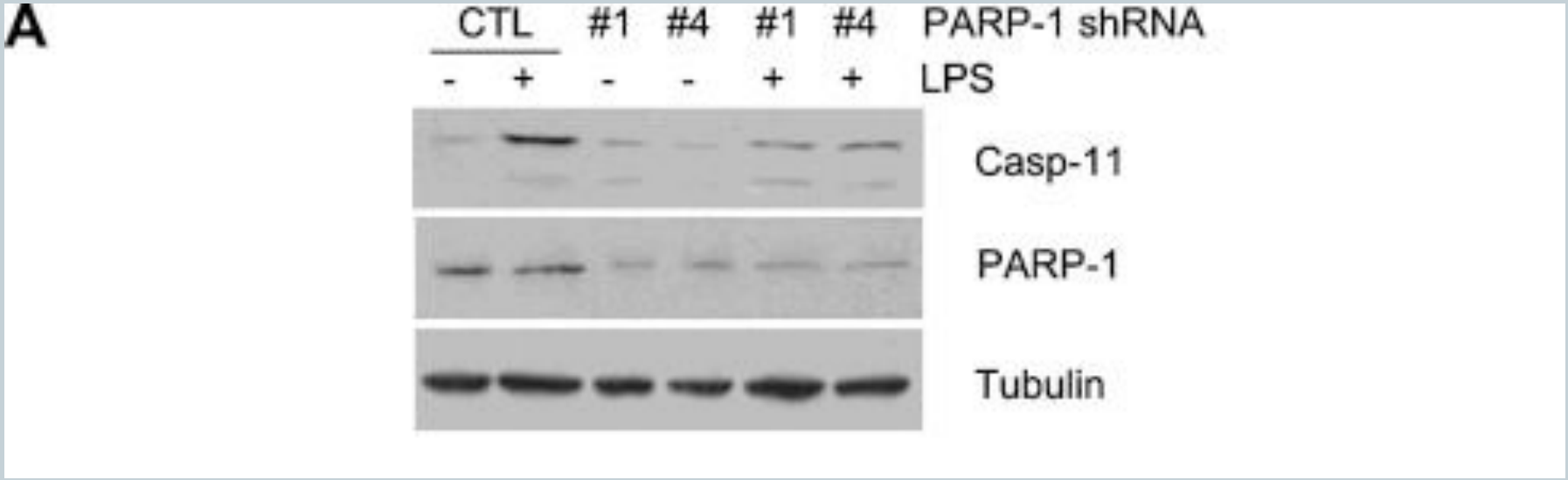
P: Examined the effect of PARP-1 knockdown on Casp-11 expression using an immunoblot assay (Western Blot).

R: When PARP-1 expressed was knocked-down, the level of caspase-11 protein was reduced compared to control knockdown.

C: Results suggest that PARP-1 is required for the LPS-induced expression of caspase-11.

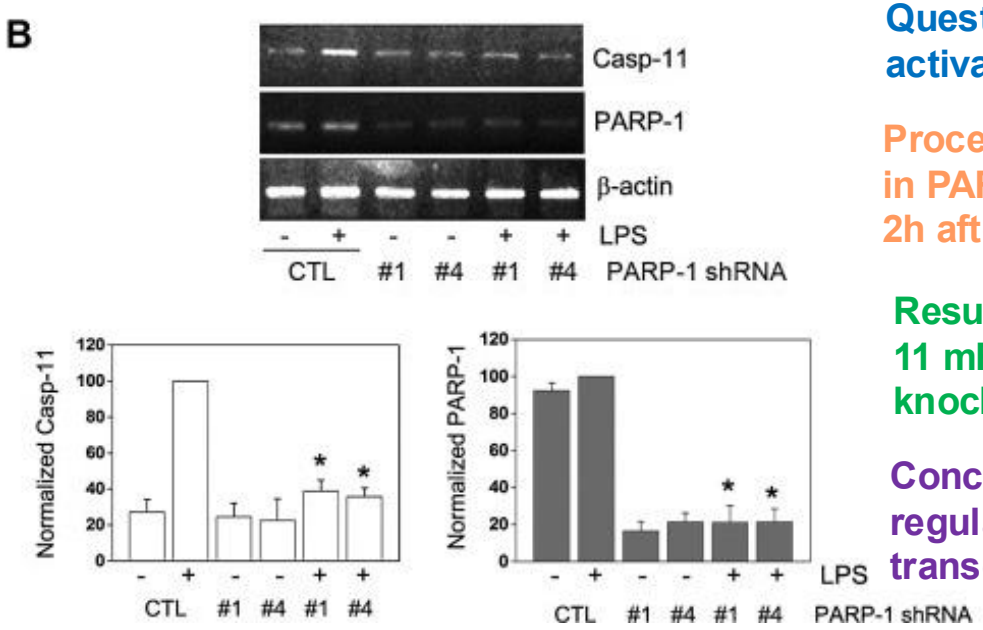
The Importance of Controls

What happens to Caspase-11 protein levels following LPS stimulation when PARP-1 is knocked down?



What kind of controls do you expect? Why are they important? How do they help you interpret your data?

What are QPRC?



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To determine whether PARP-1 is required for the transcriptional activation of caspase-11 gene, we examined the mRNA level of caspase-11 in PARP-1 knocked-down MEFs by RT-PCR at 2 h after LPS (1 μg/ml) stimulation. As shown in Fig. 1B, LPS-induced transcription of caspase-11 mRNA was also reduced by PARP-1 knockdown, suggesting that PARP-1 can regulate the induction of caspase-11 at a transcriptional level.

Question: Is PARP1 required for the transcriptional activation of caspase-11 gene?

Procedure: Examined mRNA level of caspase-11 in PARP-1 knocked-down MEFs by RT-PCR at 2h after LPS stimulation.

Results: LPS-induced transcription of caspase-11 mRNA was also reduced by PARP-1 knockdown

Conclusions: This suggests that PARP-1 can regulate the induction of caspase-11 at a transcriptional level.

What controls are being used here?

Figure 1

1. **Question it sought to answer**?

   Does PARP-1 regulate expression of Caspase-11 following LPS stmulation?

2. **Basic procedures**: Looked at mRNA (RT-PCR) and protein levels (Western Blot) of Caspase-11, with and without LPS stimulation, in the presence of control shRNA or shRNA to knockdown PARP-1

3. **Results:** Following LPS stimulation, Caspase-11 mRNA and protein was reduced 35-40% after PARP-1 knocked down

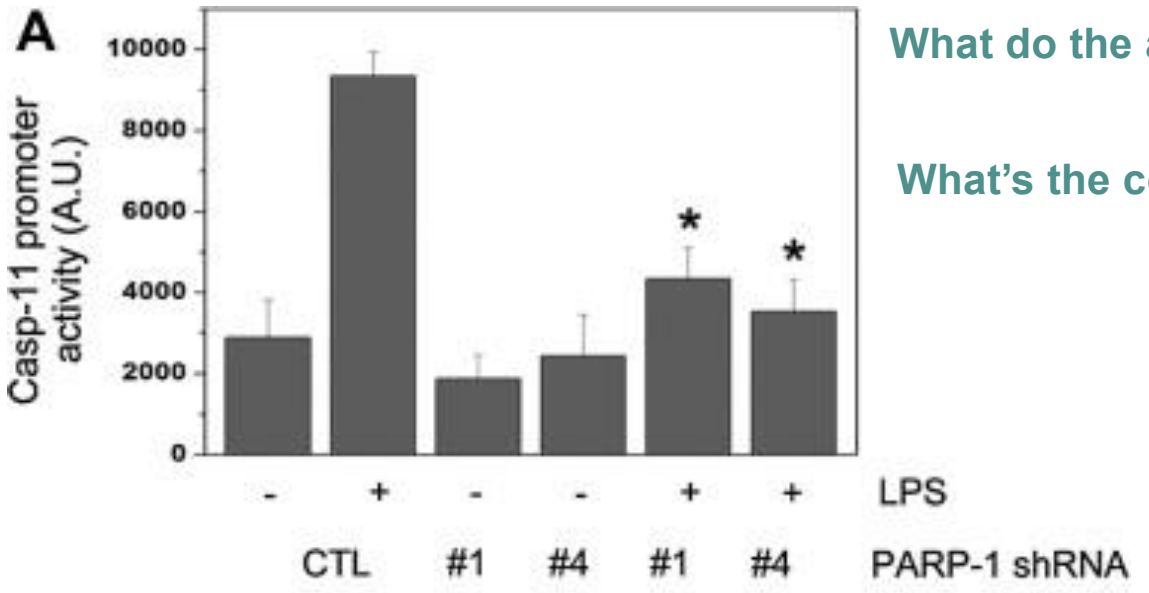
4. **Their conclusion?** (…and do you agree?)

   PARP-1 is regulating the expression of Caspase-11 at the transcriptional level.

Keep going…

Do this process for the remaining figures…

- Remember to lookup anything you don't understand
- Refer to the methods and results section as you evaluate the figures and remember to determine *QPRC* for each…
- Continue to write out notes and draw illustrations…



What do the asterisks mean?

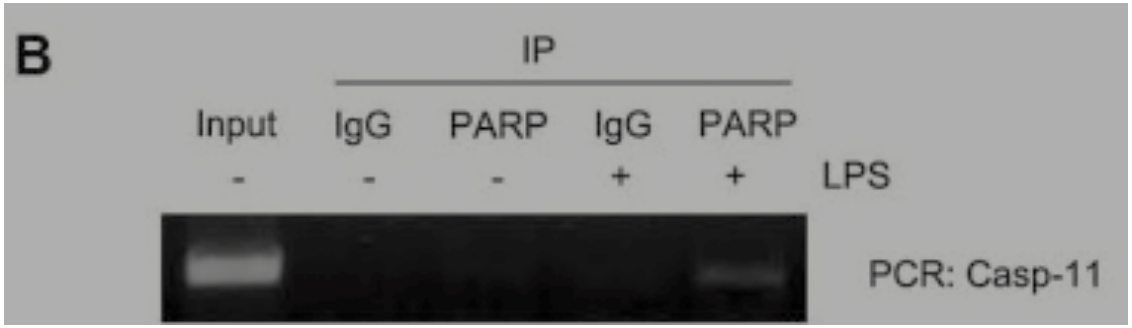
What's the control?

Question: What effect does PARP-1 knockdown have on the caspase-11 promoter **activity** following LPS stimulation?

Procedure: Transfected MEFs with caspase-11 promoter driving the firefly luciferase reporter gene and performed a dual luciferase assay.

Results: Knockdown of PARP-1 resulted in the significant reduction of LPS-induced activation of caspase-11 promoter

Conclusions: Results suggest that PARP-1 regulates the transcription of caspase-11 following LPS stimulation.



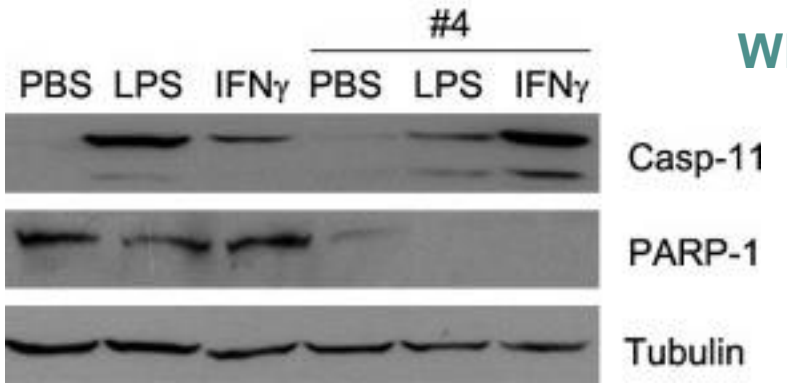
What are the controls?

Question: How does PARP-1 regulate the transcription of caspase-11? Is PARP-1 recruited to the caspase-11 promoter?

Procedure: ChIP assay to examine whether PARP-1 binds to promoter region of caspase-11

Results: The chromatin immunoprecipitated with anti-PARP-1 antibody contained caspase-11 promoter regions, but only when stimulated with LPS.

Conclusions:PARP-1 gets recruited to the caspase-11 promoter following NFKB activating stimuli.



What are the controls?

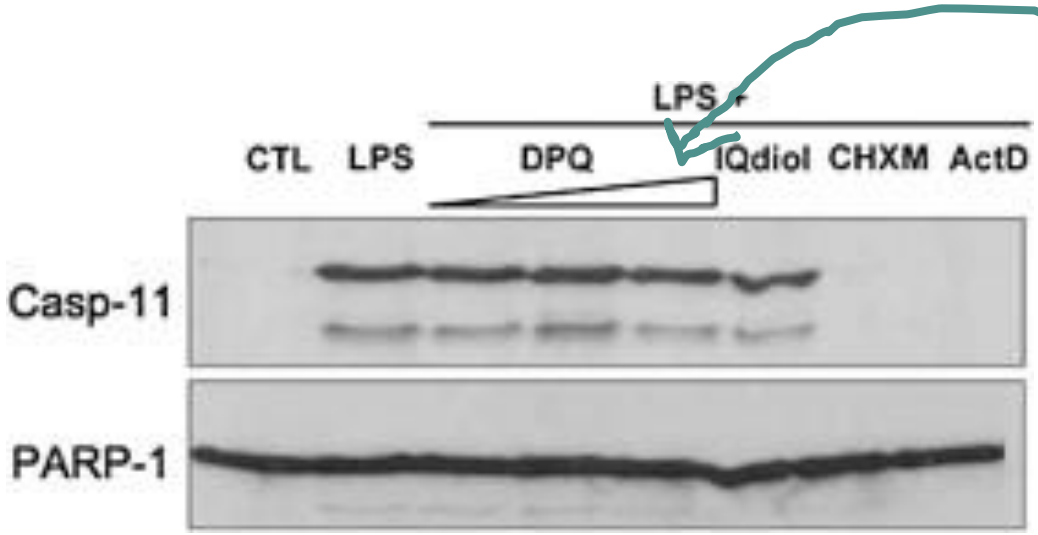
Why are they doing this? Draw a schematic to understand!

Question: If PARP-1 participates in the activation of caspase-11 promoter as a coactivator of NFKB, will knockdown of PARP1 only suppress the NFkB but not the STAT1 dependent caspase-11 expression?

Procedure: Transfect MEFs with knockdown constructs and then stimulate with LPS or IFN to activate NFkB or STAT1 pathways; Western Blot to detect protein expression.

Results: PARP-1 knockdown efficiently suppressed the caspase-11 expression induced by LPS. However, IFN induced caspase-11 expression was not suppressed by PARP-1 knockdown.

Conclusions: Results suggest that PARP-1 regulates the expression of caspase-11 as a transcriptional coactivator of NFkB



What does this triangle mean?

What are the controls?

Question: Is PARP-1 enzymatic activity required for the regulation of LPS induced caspase-11 expression?

Procedure: Stimulated cells with LPS in the presence or absence of PARP-1 inhibitors and examine levels of caspase-11

Results: Specific inhibitors of PARP-1 did not suppress the LPS induced caspase-11 expression.

Conclusions: Enzymatic activity of PARP-1 is not required for the caspase-11 inducing function of PARP-1 in MEFs following LPS stimulation.

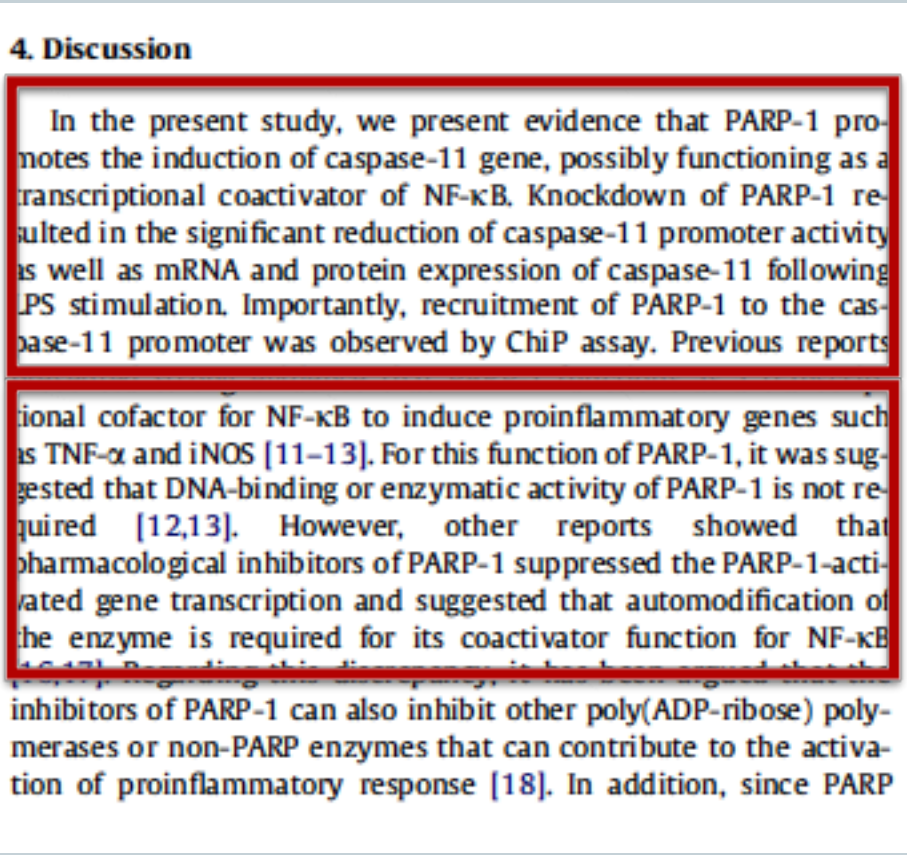
Analyze the Results

- What is the one major finding?
- Were enough of the data presented so that you feel you can judge for yourself how the experiment turned out?
- Did you see patterns or trends in the data that the author did not mention? Were there problems that were not addressed?

Now for the Discussion

- Know your conclusions before you read those of the author(s)
- Think about a trial…
  - As a juror, you should weigh the evidence and draw your own conclusions before you listen to the closing arguments… don't let others' interpretations sway your opinion
  - The only way to ensure that doesn't happen is to make sure you know what your opinion is ***before*** you hear/read the closing arguments/discussion

Discussion



4. Discussion

In the present study, we present evidence that PARP-1 promotes the induction of caspase-11 gene, possibly functioning as a transcriptional coactivator of NF-κB. Knockdown of PARP-1 resulted in the significant reduction of caspase-11 promoter activity as well as mRNA and protein expression of caspase-11 following LPS stimulation. Importantly, recruitment of PARP-1 to the caspase-11 promoter was observed by ChiP assay. Previous reports

tional cofactor for NF-κB to induce proinflammatory genes such as TNF-α and iNOS [11–13]. For this function of PARP-1, it was suggested that DNA-binding or enzymatic activity of PARP-1 is not required [12,13]. However, other reports showed that pharmacological inhibitors of PARP-1 suppressed the PARP-1-activated gene transcription and suggested that automodification of the enzyme is required for its coactivator function for NF-κB

inhibitors of PARP-1 can also inhibit other poly(ADP-ribose) polymerases or non-PARP enzymes that can contribute to the activation of proinflammatory response [18]. In addition, since PARP

- In the Discussion…
  - Data is analyzed to show what the authors believe the data show. You don't have to agree with their interpretations!
  - Findings are related to other findings in the field (contribute to knowledge, correct errors, etc.)– How is this work significant?

Analyze the Discussion

- Do you agree with the conclusions drawn from the data?
- Are these conclusions over-generalized or appropriately careful?
- Are there other factors that could have influenced, or accounted for, the results?
- What further experiments would you think of, to continue the research or to answer remaining questions?

In the end, you should ask and be able to answer the following…

- What ***questions*** does the paper address?
- What are the main ***conclusions*** of the paper?
- What ***evidence*** supports those conclusions?
- Do the data actually ***support*** the conclusions?
- What is the ***quality*** of the evidence?
- Why are the ***conclusions*** important?

Reflection and Criticism

- Did the authors meet your expectations?
- Do you agree with the authors' rationale for setting up the experiments as they did?
- Did they perform the experiments appropriately? (Repeated a number of times, used correct control groups, used appropriate measurements etc)

Final thoughts

- Reading a paper will probably take SEVERAL hours
- Successfully analyzing research articles will not happen overnight
- Keep working to ***UNDERSTAND***