

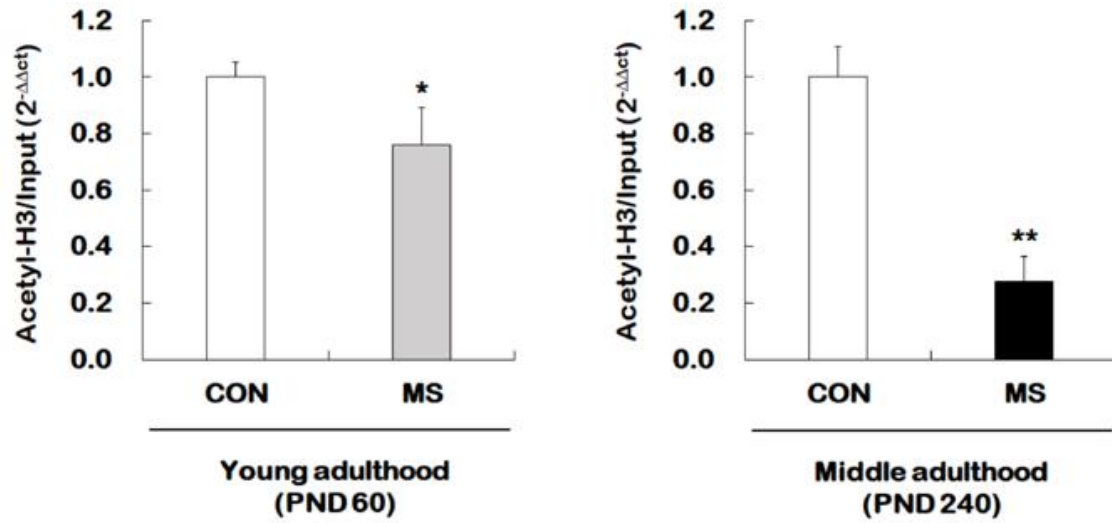
Part 1 (35 points)

Refer to the paper “Effects of Early Life Stress on Epigenetic Changes of the Glucocorticoid

Receptor 1 γ Promoter during Adulthood” by Seo et al

Figures 4 and 5 from the paper are included below.

A.



B.

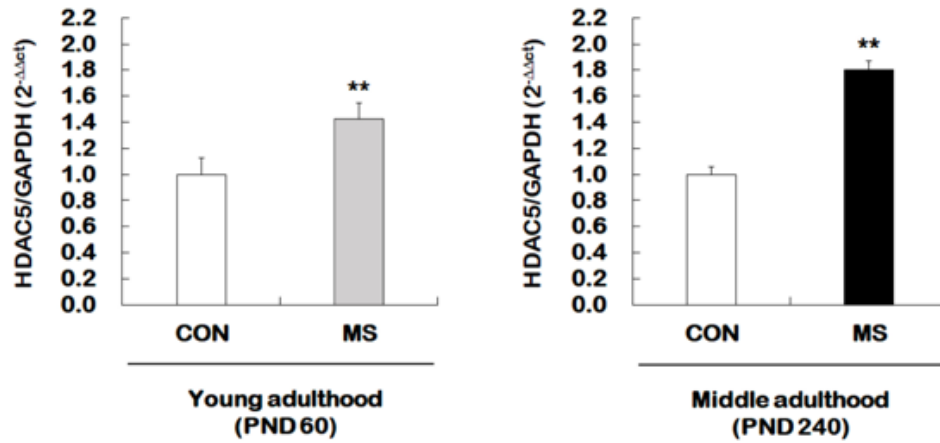
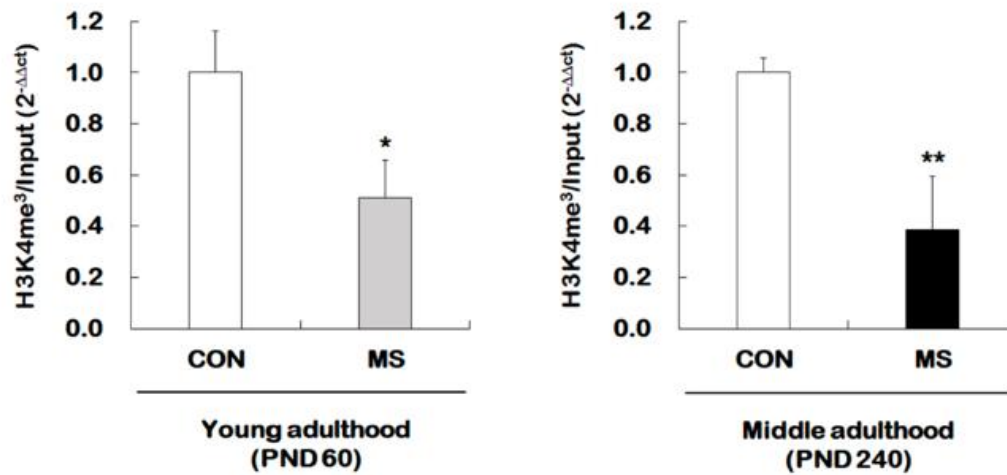


Figure 4. Age-dependent changes in the level of histone H3 acetylation at the GR promoter 1₇ and HDAC5 expression following MS exposure. (A) ChIP assays were used to measure the level of acetylated histone H3 (Acetyl-H3) at the GR promoter 1₇ in the hippocampus using an antibody to Acetyl-H3. Data were normalized to input DNA (*n* = 10–13/group). (B) The HDAC5 mRNA level in the hippocampus was measured using qRT-PCR. All quantities were normalized to GAPDH (*n* = 9–13/group). These levels were analyzed in young (PND 60) and middle (PND 240) adulthood, respectively. Data were expressed as values relative to the CON group using the 2^{-ΔΔCt} method and represent the mean ± SEM (* *p* < 0.05, ** *p* < 0.01, unpaired Student t-test).

A.



B.

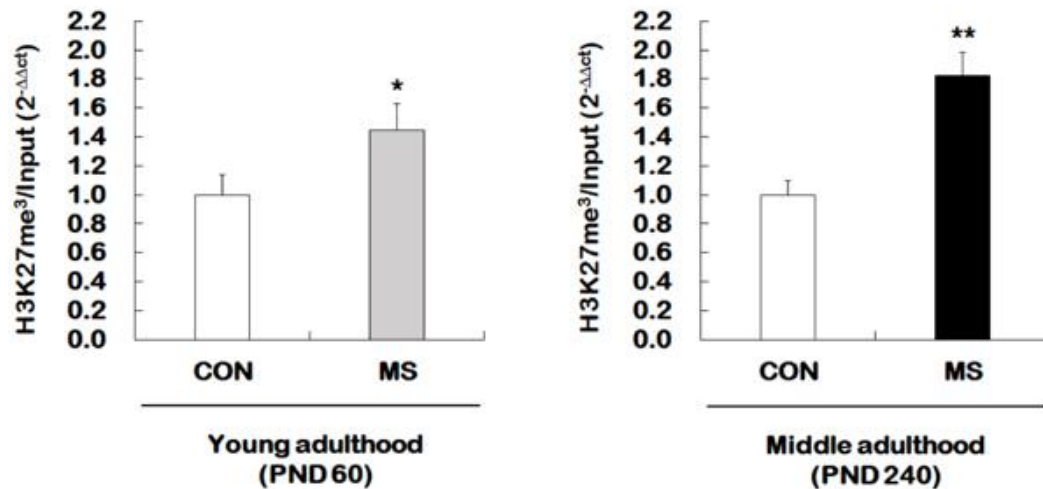


Figure 5. Age-dependent changes in the levels of histone H3 methylation at the GR promoter 17 following MS exposure. ChIP assays were used to measure the level of acetylated histone H3 trimethylated K4 (H3K4me³, A) and K27 (H3K27me³, B) at GR promoter 17 in the hippocampus using antibodies to H3K4me³ and H3K27me³. These levels were analyzed in young (PND 60) and middle (PND 240) adulthood, respectively. Data were normalized to input DNA and are expressed as values relative to the CON group using the 2^{-ΔΔCt} method, and represent mean ± SEM (*n* = 9–13/group, * *p* < 0.05, ** *p* < 0.01, unpaired Student *t*-test).

Questions

- 1) Describe the results that are being shown in Figure 4 and 5 (separate descriptions for A and B). Include in your answer: what experiment is being done, what is being compared (state the control) and what specifically was being measured. State exactly the probe(s) used, or method of detection (state what specifically was needed as part of the experiment. You may need to refer to the materials and methods). State what the data in the histogram represents. Be as clear as possible, stating only the unique/specific components of this experiment without listing reagents that are common to all experiments of the same type. (20)
- 2) Briefly state what the results imply, that is, what impact the results in figures 4 and 5 would have on the gene in each case, and also on the animal in later life. (5)

- 3) Compare the assay in Figures 4A and 5 A&B to the same assay in the paper by Weaver et al. What are the similarities and differences between what was found in the Weaver paper vs this one with respect to this assay? Be specific in terms of the treatment and probes used. (3)
- 4) Speculate on what the purpose could be of the effect being observed specifically at this 17 promoter. What do the authors mean when they refer to the 17 promoter? You do not need to include specific details about the promoter, but comment on what has been observed in this paper and the Weaver paper and comment on what impact this might have on the regulation of the gene for the glucocorticoid receptor. (4)
- 5) Explain why the experiments were important to understand the type of interaction that is taking place in this study, based on their hypothesis. What study would you add? (3)

Part 2 (33 points)

Refer to the paper: "*Glucocorticoid receptor signaling represses the antioxidant response by inhibiting histone acetylation mediated by the transcriptional activator NRF2*" by Alam et al

Dexamethasone and betamethasone are ligands (drugs that bind to a receptor) for the glucocorticoid receptor (GR). That is, they are drugs which induce the translocation of GR from the cytosol to the nucleus, through their binding. GR is a ligand-bound transcription factor, which can activate or inhibit transcription subsequent to ligand binding.

Diethyl maleate (DEM) is used to induced expression of representative NRF2 target genes in the study (positive stimulation of transcription).

A knockdown approach using siRNA is d escribed in the paper. This approach is used to reduce the amount of a protein in a specific cell by introducing single interfering RNAs in the cell These are highly specific sequences designed to target a particular gene they work similar to the way microRNA works, but are more specific to a single RNA target and are therefore used experimentally.

Figures from the paper follow:

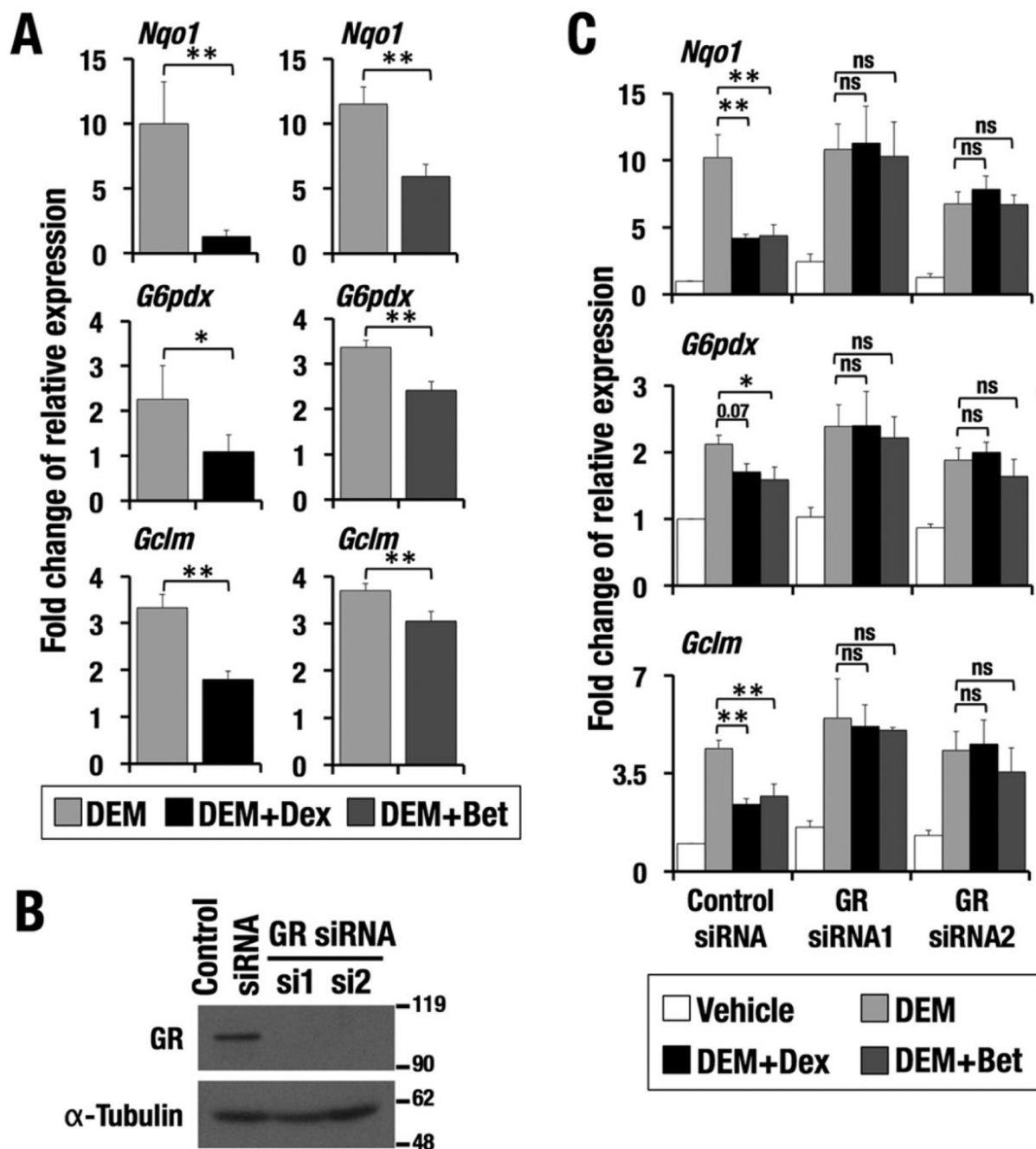


Figure 2. GR represses inducible expression of NRF2 target genes in response to DEM. A, relative expression levels of NRF2 target genes in Hepa1c1c7 cells treated with DEM, DEM + Dex, and DEM + Bet. B, protein levels of GR in Hepa1c1c7 cells with or without GR knockdown. C, relative expression levels of NRF2 target genes in Hepa1c1c7 cells with or without GR knockdown. Cells were treated with DEM, DEM + Dex, and DEM + Bet. -Fold changes were calculated against vehicle-treated samples, and the average and S.D. (error bars) of the -fold changes from three independent experiments are shown (A and C). Student's *t* test (A) and one-way ANOVA and Tukey's post hoc test (C) were performed as statistical analyses. *, $p < 0.05$; **, $p < 0.01$; ns, nonsignificant.

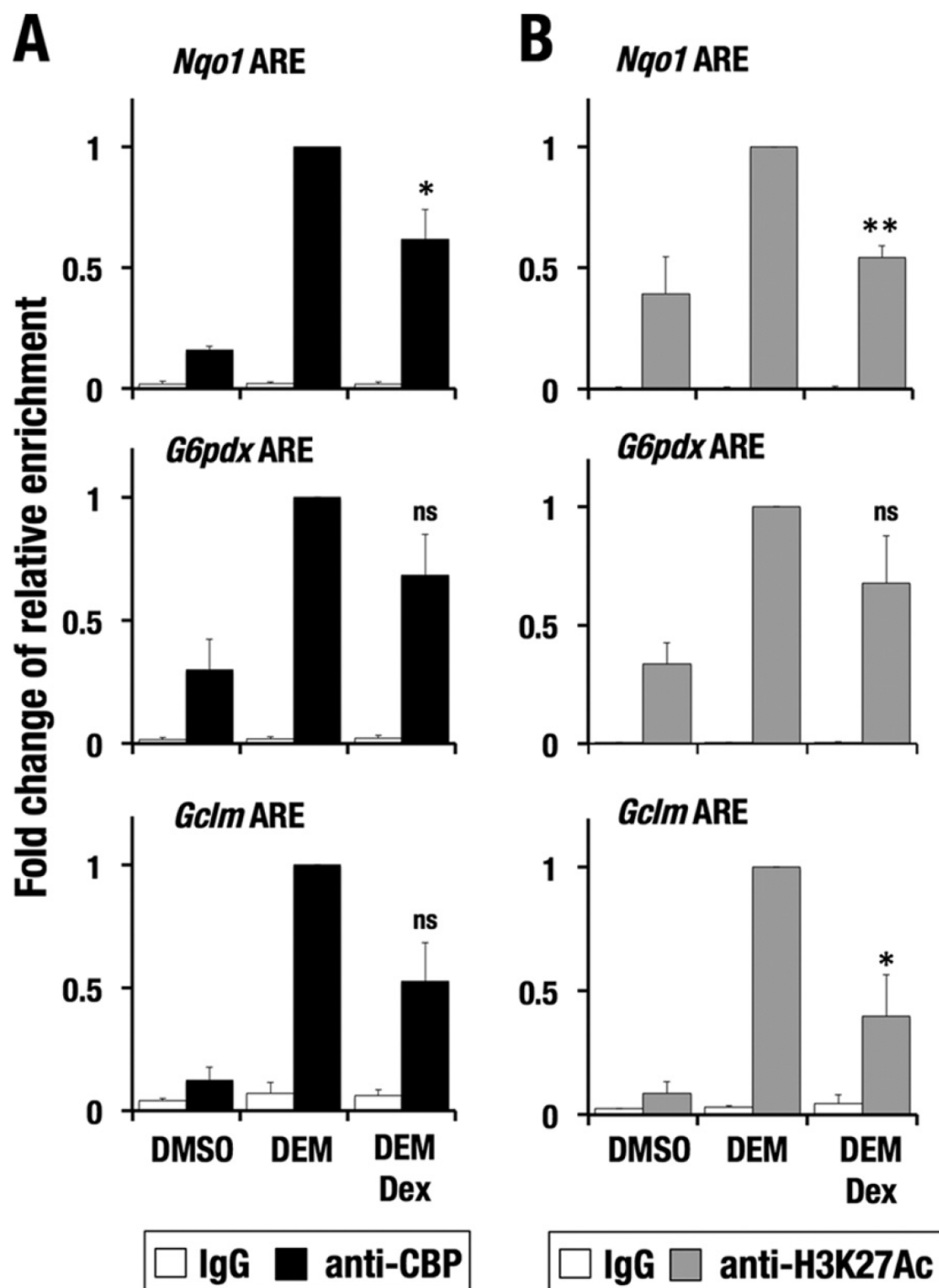


Figure 8. Dex treatment reduces CBP recruitment and H3K27 acetylation at AREs. A and B, ChIP assay with anti-CBP (A) and anti-H3K27Ac (B) antibodies using Hepa1c1c7 cells treated with DEM and DEM + Dex. -Fold changes of relative enrichments for DEM + Dex-treated and vehicle-treated cells against DEM-treated cells were calculated. The average and S.D. (error bars) of the -fold changes from triplicate reactions are shown. Confidence interval estimation was conducted for DEM + Dex-treated samples. *, $\alpha < 0.05$; **, $\alpha < 0.01$; ns, nonsignificant.

Questions:

- 1) In your own words, describe the conditions in which the cell is normally able to increase levels of NRF2. What specific factor is regulated, and how? What benefit is this to the cell? (7)
- 2) In this study, how was NRF2 isolated? Describe the process briefly. (3)
- 3) Small interfering RNA (siRNA) works in a similar way to microRNA (miRNA) but is more specific at being able to target specific genes. Describe the results shown in figure 2 and why they are important. (10)
- 4) Describe briefly how GR was found to be interacting with the actions of NRF2 in these cells (describe two mechanisms). How was this shown? Be as concise as possible. How does this differ from the other ways in which GR regulates transcription? Give two other examples of how GR can otherwise regulate transcription that do not involve NRF2. (9)
- 5) In Figure 8, above, describe the assay that is being done. What is the significance of what is being shown? In your answer, explain why the authors used the antibodies they did. What do the six graphs within the figure represent? What does the figure tell us about the actions of the glucocorticoid receptor and why? (4)

Part 3A (32 points)

540 Dumas et al.

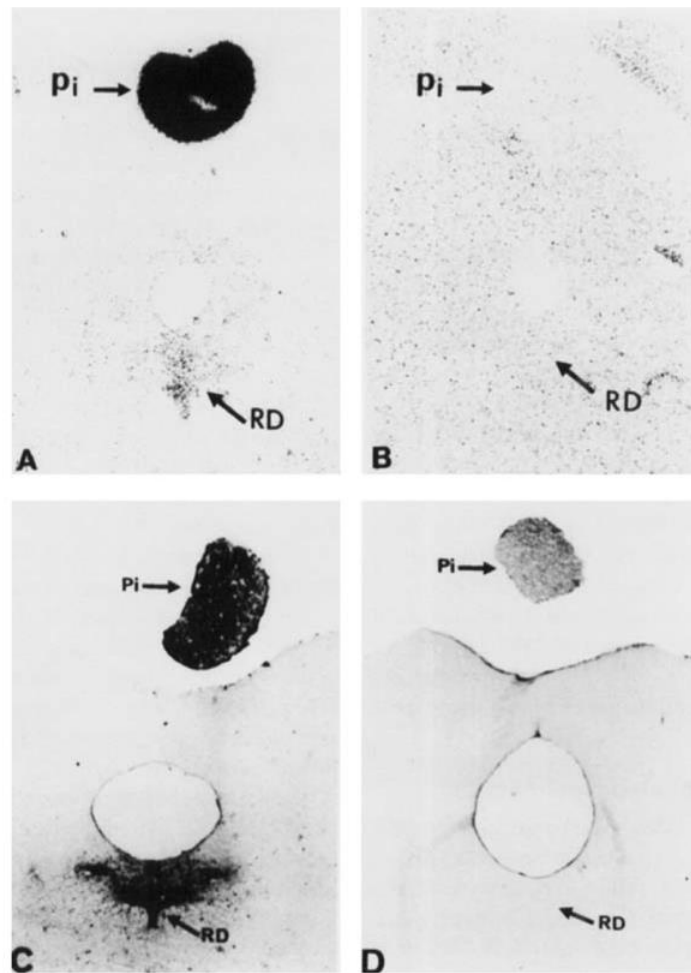


Fig. 1. Comparative reactivity of TPH antibodies and cDNA probe on tissue sections. First, adjacent frontal sections of brain stem were hybridized with (A) a probe corresponding to the coding region and (B) a control pBR322 probe. (Magnifi-

cation: $15\times$). Second, similar adjacent sections were treated with (C) affinity-purified TPH antibody ($0.2\text{ }\mu\text{g/ml}$) and (D) pre-immune serum as control ($1/500$). (Magnification $15\times$). Pi: pineal gland; RD: raphe dorsalis.

The data above, taken from Dumas et al (1989) shows that a radioactive cDNA probe for the TPH gene coding region strongly labels the pineal gland (Pi), but not the raphe dorsalis, RD (A). In the same tissue (an adjacent slice) a nonsense probe shows no binding (B). In frame C, TPH antibody labels both the pineal and the raphe dorsalis strongly, while the control serum (no recognition for TPH) shows no binding (D).

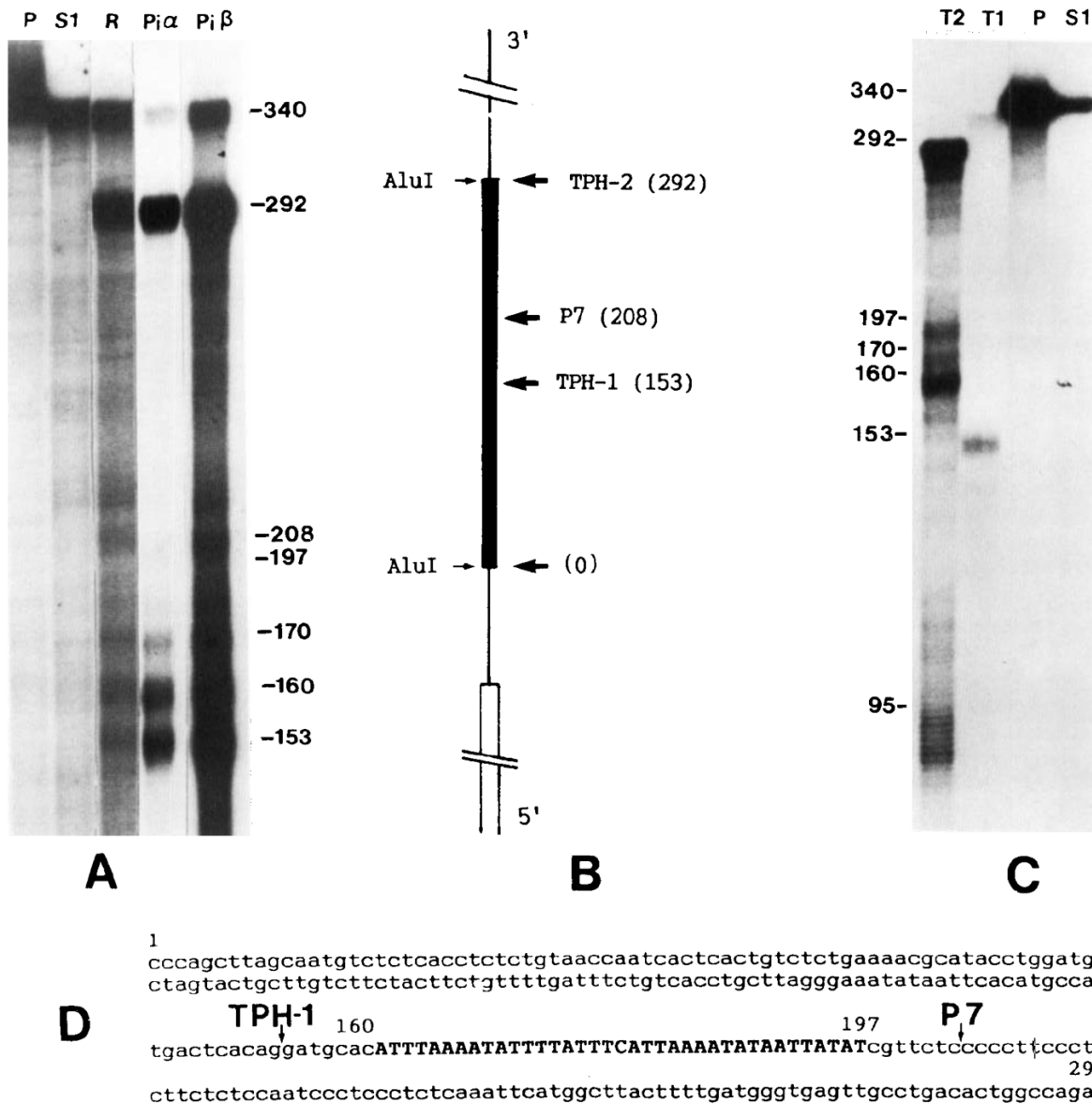


Fig. 3. S1 nuclease mapping analysis of the 3' untranslated region of TPH mRNAs. **A:** S1 nuclease mapping with a probe containing 153 bases common to TPH-1 and 2 mRNAs, plus 139 bases unique to TPH-2. **Lane P:** Undigested probe; **lane S1:** 40 μ g of yeast transfer RNA; **lane R:** 10 μ g raphe poly(A)⁺ RNA (ten dissected raphe regions); **lanes Piα and Piβ:** 1 μ g of pineal gland RNA (0.5 pineal gland). Exposure times were 15 days for all lanes except for one exposure of Pi (Piα) for 1 day, which gave fainter signals. The lengths of the

protected fragments are indicated. **B:** Map of the 3' UTR of the TPH mRNAs. The black box corresponds to the probe used. The 3' end of the different TPH mRNAs are indicated. The white box indicates the 3' end of the coding sequence. **C:** S1 nuclease mapping with synthetic TPH mRNAs. **Lane T2:** 10 ng of TPH-2 mRNA; **lane T1:** 10 ng of TPH-1 mRNA; **lane P:** undigested probe; **lane S1:** 40 μ g of yeast transfer RNA. **D:** Sequence of the 3' UTR probe. The A-T-rich putative cleavage region is represented in bold type.

The sequence shown in figure 3 corresponds to a sequence in the rat TPH1-201 (3897 bp) transcript on ensemble.org that begins on the 94th base pair into the 3'UTR

Questions:

- 1) State which probe was described for use in the *in situ* hybridization in the paper by Dumas (1)
- 2) How are the histology result in this paper surprising? How do the authors explain the difference in the *in situ* hybridization vs the immunohistochemistry results? (2)
- 3) Explain on the level of cell biology (on a molecular level) what could lead to a tissue-specific discrepancy such as this in a wild-type (unmutated) animal? Describe the factors involved. (4) *Note, please describe the mechanism behind the authors' (Dumas et al) conclusions.
- 4) How did the different mRNA molecules in the Dumas paper differ from each other? Describe the technique used to identify these molecules. (4)
- 5) Describe the enzymes and other factors involved in creating the transcripts that were identified. Using the ensemble sequence for the rat TPH1 gene, what sequence would you look for and in which region of the gene to explain the multiple transcripts? Can you find a sequence that matches the description? Present a figure showing a representative sequence in the rat TPH1 gene which would help to explain the existence of various sized mRNA transcripts. Give reasoning for your choice. (11)

Refer to the paper "*Differential tissue distribution of tryptophan hydroxylase isoforms 1 and 2 as revealed with monospecific antibodies*" by Sakowski et al.

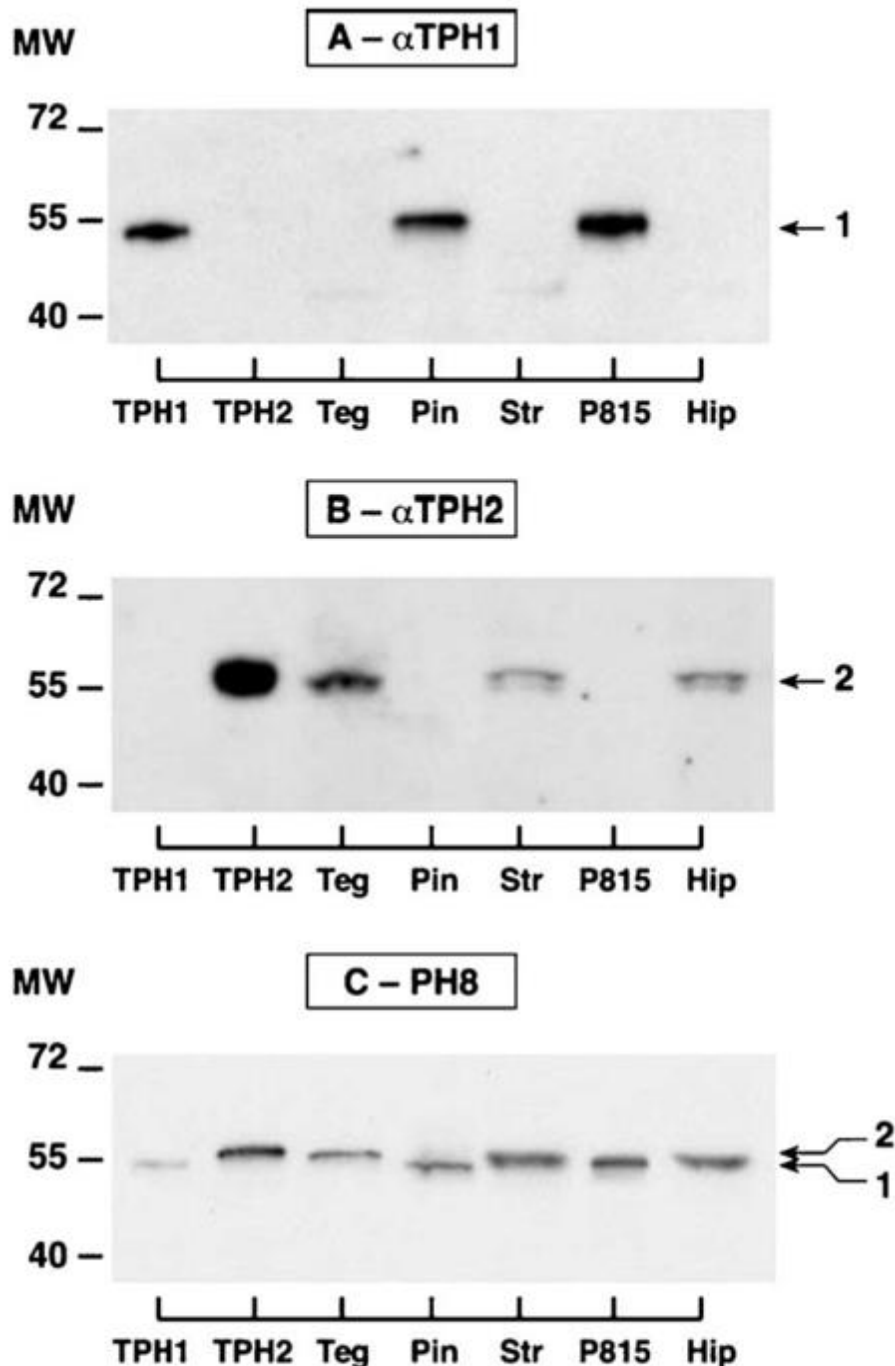


Fig. 2 – Immunoblotting of TPH1 and TPH2 in mouse tissues. Soluble extracts from mouse mesencephalic tegmentum (Teg, 30 μ g), pineal (Pin, 20 μ g), striatum (Str, 30 μ g), P815 mastocytoma cells (P815, 5 μ g), or hippocampus (Hip, 30 μ g) were resolved by SDS-PAGE and blotted to nitrocellulose. Blots were probed with α TPH1 (A, 1:1000), α TPH2 (B, 1:1000) or PH8 (C, 1:20,000) overnight at 4 °C. After exposure to horseradish peroxidase-conjugated secondary antibodies (1:4000) for 1 h at room temperature, blots were washed, and TPH immunoreactivity was visualized by enhanced chemiluminescence. Lanes 1 and 2 of each panel contain

Questions cont:

6) Note, you might be confused with the fact that the terms TPH-1 (TPH1) and TPH-2 (TPH2) are used in both papers, however they do not refer to the same thing. What is the difference? (2)

7) Pay careful attention to publication dates. Using the results of Sakowski, and references therein, explain how the Dumas paper was misinterpreted by the authors and why. What experiments could the Dumas group have done sooner in order to reach a different conclusion. Give two examples. (8)