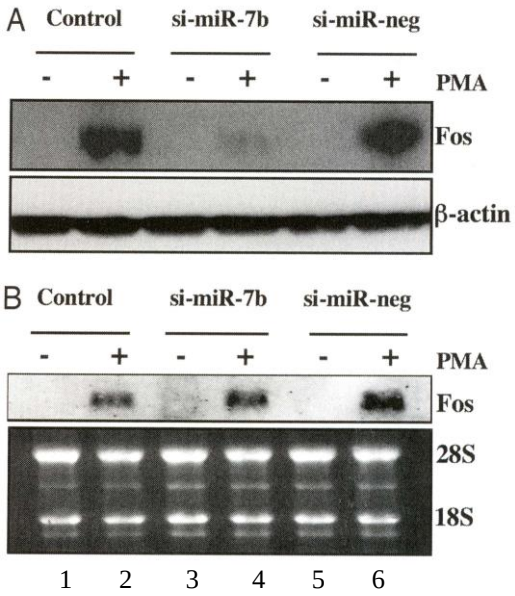
THE ROLE OF A MICRO-RNA IN THE REGULATION OF FOS GENE EXRESSION

Terms to be familiar with before you start to analyze the figure

*micro-RNAs * Fos protein * gene expression * AP1 transcription factor* cloning * expression vector * plasmid * transfection * phorbol ester * Western blotting * Northern blotting * ethidium bromide staining*

The figure



The results presented in the figure come from an experiment in which the role of miR-7b micro-RNA was studied. miR-7b shows partial sequence complementarity to the 3'-untranslated region of *fos*-mRNA. (The Fos proto-oncogenic protein is a component of the AP1 transcription factor, an important regulator of gene expression.) A DNA fragment coding for miR-7b RNA, and another fragment coding for a micro-RNA unrelated to *fos*-mRNA were cloned into expression vectors, and the plamids (designated si-miR-7b, samples 3 and 4, and si-miR-neg, samples 5 and 6, in the figure) were transfected into mouse fibroblasts, non-transfected cells were used as controls (samples 1 and 2). Some of the cultures were treated with a phorbol ester (PMA; samples 2, 4 and 6), while others were left untreated (samples 1, 3 and 5).

A. 2 hours after PMA treatment protein extracts were prepared from the cultures and were then subjected to Western blot analysis using anti-Fos (upper panel) and anti-actin (lower panel) antibodies.

B. RNA was isolated from the cells after 1 hour PMA treatment, subjected to formaldehyde/agarose gel electrophoresis and ethidium bromide staining (lower panel) followed by Northern blotting using a *fos* cDNA probe (upper panel).

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Answer the following questions:

1. What was the aim of using anti-β-actin antibody (panel A)?
2. What was the aim of staining the agarose gel with ethidium bromide (panel B)?
3. What was the aim of expressing miR-neg in the cells?
4. What was the effect of phorbol ester treatment? What signaling protein mediated this effect?
5. Which phase of gene expression was affected by the micro-RNA?

The source of the figure

Lee, H-J., Palkovits, M., Scott, Y.W. (2006) MiR-7b, a micro-RNA up-regulated in the hypothalamus after chronic hyperosmolar stimulation inhibits Fos translation. Proc. Nat. Acad. Sci. USA *103*, 15669-15674.

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