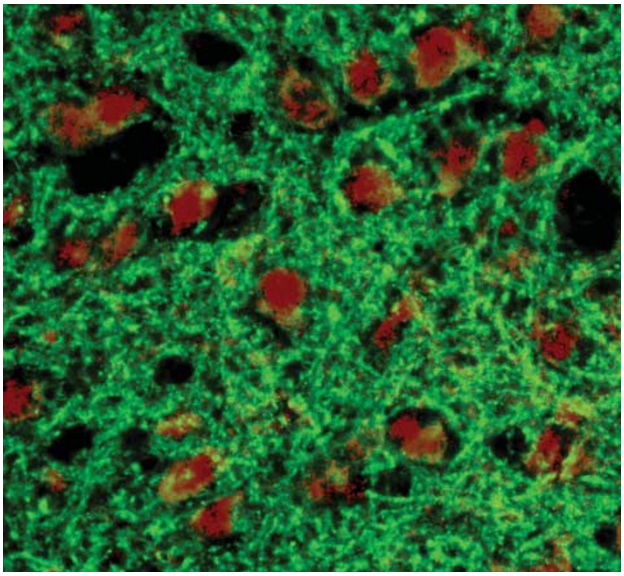
NEUROBIOLOGY OF ADDICTION

The rewards of tPA



Immunohistochemical localization of MAP2 (green) and tPA (red) in morphine-treated mice. Image courtesy of K. Yamada, Kanazawa University Graduate School of Natural Science & Technology, Japan.

The tissue plasminogen activator (tPA)–plasmin system, which degrades extracellular matrix proteins, regulates the brain's response to morphine, according to new work by Nagai *et al.* Writing in *The Proceedings of the National Academy of Sciences of the USA*, they show that this system regulates the morphine-induced release of dopamine in the nucleus accumbens, which is thought to be involved in morphine's rewarding effects.

The extracellular protease tPA converts plasminogen into plasmin, and is expressed throughout the CNS. The tPA–plasmin system is thought to be important for various neuronal functions, including plasticity, migration and neurite outgrowth. Nagai and colleagues investigated the response of this system to treatment with morphine in rats and mice, and found that morphine treatment led to an increase in the expression of tPA in the nucleus accumbens, and that this effect was blocked by naloxone (an opioid receptor blocker).

They then investigated the effects of morphine in mice that lacked either tPA or plasminogen. Although the anti-nociceptive effects of morphine were normal in these mice, they showed much less morphine-induced hyperactivity than wild-type mice. This deficit could be partly reversed by injecting tPA or plasmin into the nucleus accumbens. In addition, the mutant mice failed to develop normal conditioned place preference following morphine treatment, and this is generally considered to be a measure of the rewarding properties of morphine. However, the interpretation of this finding is complicated by the fact that tPA-knockout mice also show deficits in other measures of learning.

If the tPA–plasmin system is necessary for the rewarding effects of morphine, what is its role? Normally, morphine causes a release of dopamine in the nucleus accumbens, and this is related to the rewarding effects of morphine. In mice that lacked tPA or plasminogen, the amount of

DEVELOPMENT

RNA snippets specify cell fate

A new study in *Cell* shows that multipotent stem cells are committed to a neuronal lineage through the novel regulatory action of small, double-stranded RNAs (dsRNAs).

Small RNAs have received a lot of attention recently owing to their involvement in RNA interference, the process by which snippets of RNA bind specifically to target mRNAs and prevent their translation into protein. Now, Fred Gage and colleagues have discovered a new mode of action of dsRNAs — activating gene expression by interacting directly with proteins and DNA.

The discovery was made while investigating the molecular mechanisms that regulate neuron-specific gene expression. In a screen for small, non-coding RNAs that might participate in the differentiation of neurons, Gage's team isolated a short dsRNA whose sequence matched that of *neuron restrictive silencer element/RE1* (*NRSE/RE1*) from adult hippocampal neural stem cells. *NRSE/RE1* is a conserved DNA response element that is present in genes that code for neuronal proteins, including ion channels and neurotransmitter receptors. In non-neuronal cells, expression of neuronal genes is prevented when the *NRSE/RE1* element in their promoters binds to neuronal restricted silencing factor/RE1 silencing transcription factor (NRSF/REST). This zinc finger protein mediates its repressive effect on gene expression by recruiting negative transcriptional regulators such as histone deacetylases.

The authors used viral vectors to introduce *NRSE/RE1* dsRNAs into adult hippocampal stem cells. This transfection caused morphological changes consistent with the differentiation of neurons, such as the extension of processes. The expression of neuron-specific genes that contain *NRSE/RE1* in their promoters (including *synapsin 1* and *mGluR2*) increased in these cells. So *NRSE/RE1* dsRNAs seem to have a crucial role in the acquistion of neuronal cell fate by counteracting the repressive action of NRSF/REST.

Do *NRSE/RE1* dsRNAs exert their effect by silencing the *NRSF/REST* gene through RNA interference? As expression of NRSF/REST was unaffected in cells transfected with *NRSE/RE1* dsRNAs, the authors concluded that this was not the case. Support for an alternative mechanism came from experiments in which stem cell extracts were incubated with biotin-labelled *NRSE/RE1* dsRNAs. Immunoblots of biotin-positive conjugates and titration analysis revealed that *NRSE/RE1* dsRNA binds NRSF/REST in a highly-specific manner.

So, a model is emerging whereby cells that are to become neurons activate the transcription of genes that contain the *NRSE/RE1* sequence. These cells might then generate non-coding *NRSE/RE1* dsRNAs that interact with the *NRSE/RE1* DNA response element and NRSF/REST, switching this transcription factor from repressor to activator by disrupting its association with negative transcriptional regulators. No doubt many more examples of transcriptional regulation by other small modulatory RNAs of this type will come to light in the near future.

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References and links

ORIGINAL RESEARCH PAPER Kuwabara, T. *et al.* A small modulatory dsRNA specifies the fate of adult neural stem cells. *Cell* **116**, 779–793 (2004).

FURTHER READING Livesey, F. J. & Cepko, C. L. Vertebrate neural cell-fate determination: lessons from the retina. *Nature Rev. Neurosci.* **2**, 109–118 (2001).