Neural-Inducing Factors Produced by PA6 Stromal Cells

(A and B) NCAM staining of ES cell colonies grown from a single cell for 8 days on MEF and PA6 feeder cells, respectively. Inset, phase contrast view.

(C and D) PA6-induced ES colonies double-stained with TuJ and nestin antibodies, respectively.

(E) The presynapse-specific marker synaptophysin (Syn, green) was detected on the induced neurons (TuJ, red).

(F) Percentages of TuJ-positive ES cell colonies on gelatin, PA6, and PFA-fixed PA6 after 8 days of induction.

(G) TuJ staining of ES cells cultured on PFA-fixed PA6 for 8 days.

(H) PA6 cells induce the neuronal marker TuJ in cocultured ES cells even when separated by a filter membrane (filter). The medium conditioned with PA6 for 3 days (CM) did not induce significant neural differentiation in ES cells cultured on gelatin. Arrows, negative colonies in immunostaining.

Kawasaki et al., 2000
**Shh RNA**

**Shh protein**

**Graded Shh activity and ventral neural tube patterning**

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Sox1 – Panneural progenitor marker

Otx2 – Midbrain marker (w/En1)

Hoxc6 – Spinal cord marker

NeuN – Neuronal marker

TuJ1 – Neural specific beta tubulin
Increasing Hh-Ag1.3 (RA present in all)

Endogenous expression patterns
HB9 – MN marker
Lhx3/Is11/Lim1 – expressed by subsets of MNs
NF – Neurofilament expression

mLim2 – mouse specific interneuron marker

Lhx3 – MN marker

mNCAM – mouse spec. interneuron marker

cSC1 – Chick spec. MN marker
VAChT/Syb/Syn – nerve terminal differentiation markers

aBTX – motor axon terminal marker