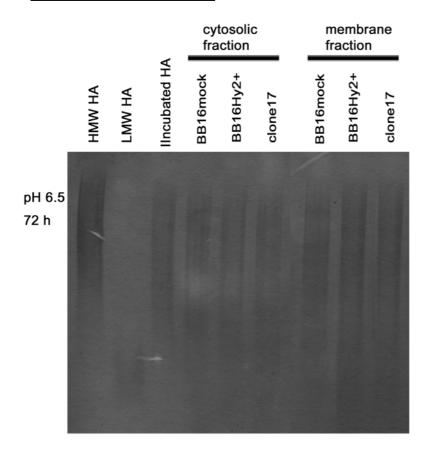


Correlation between Hyal2 expression and coat thickness in various clones. (A) Coats were visualized using a particle exclusion assay and expressed as the ratio of combined cell and pericellular matrix areas relative to the cell area. A value of 1.0 means no discernible coat. Results are presented as means \pm SEM of 20 cells in each group. All differences *vs* BB16mock are significant (P<0.05, ANOVA followed by Dunnett's multiple comparison test) except for clone 5. (B) Hyal2 mRNA was quantified using real-time RT-PCR in the same clones. Relative levels of expression are shown, using 1.0 as the Hyal2 mRNA level in BB16mock cells. (C) The amount of Hyal2 protein was evaluated using anti-Hyal2 antibodies. Actin is shown as a control in each clone. (D) Correlation between coat thickness and Hyal2 mRNA levels in the various clones indicated in A-B ($r^2 = 0.895$; P<0.0001).



Measurement of HA-degrading activity in cytosolic and membrane fractions of BB16mock, BB16Hy2+, and clone17 cells, as indicated. The conditions of preparation of these fractions as well as the incubation conditions (72 h, pH 6.5) are identical to those described by Harada, H., and Takahashi, M. (2007) *J. Biol. Chem.* **282**, 5597-5607, except that in the current experiments 282 μ g of proteins from cell extracts were incubated with 200 μ g HA. HA-degrading activity was assessed on agarose gels as described in Fig. 1D. Non-incubated HA standards of high MW (HMW HA, 2.5 x 10⁶ Da) and lower MW (LMW HA, 4 x 10⁴ Da) were loaded in the first two lanes, respectively. The third lane contains HMW HA incubated without cell extract for 72 h at pH 6.5. In comparison with that lane, no significant HA-degrading activity was detected in any cell fraction. Identical results were obtained when the experiment was repeated at pH 4.0 and when the incubation was limited to 24 h, both at pH 4.0 and 6.5.