The secretion of lung surfactant in alveolar type II cells is a complex process involving the fusion of lamellar bodies with the plasma membrane. This process is somewhat different from the exocytosis of hormones and neurotransmitters. For example, it is a relatively slower process, and lamellar bodies are very large vesicles with a diameter of approximately 1 microm. SNARE proteins are the conserved molecular machinery of exocytosis in the majority of secretory cells. However, their involvement in surfactant secretion has not been reported. Here, we showed that syntaxin 2 and SNAP-23 are expressed in alveolar type II cells. Both proteins are associated with the plasma membrane, and to some degree with lamellar bodies. An antisense oligonucleotide complementary to syntaxin 2 decreased its mRNA and protein levels. The same oligonucleotide also inhibited surfactant secretion, independent of secretagogues. A peptide derived from the N-terminus of syntaxin 2 or the C-terminus of SNAP-23 significantly inhibited Ca(2+)- and GTPgammaS-stimulated surfactant secretion from permeabilized type II cells in a dose-dependent manner. Furthermore, introduction of anti-syntaxin 2 or anti-SNAP-23 antibodies into permeabilized type II cells also inhibited surfactant release. Our results suggest that syntaxin 2 and SNAP-23 are required for regulated surfactant secretion.