Gene-specific, age-dependent regulations are common at the transcriptional and translational levels, while protein transport into organelles is generally thought to be constitutive. Here we report a new level of differential age-dependent regulation and show that chloroplast proteins are divided into three age-selective groups: group I proteins have a higher import efficiency into younger chloroplasts, import of group II proteins is nearly independent of chloroplast age, and group III proteins are preferentially imported into older chloroplasts. The age-selective signal is located within the transit peptide of each protein. Two consecutive positive charges define the necessary motif in group III signals for older chloroplast preference. We further show that different members of a gene family often belong to different age-selective groups due to sequence differences in their transit peptides. These results indicate that organelle-targeting signal peptides are part of cells’ differential age-dependent regulation network. The sequence diversity of some organelle-targeting peptides is not a result of lacking selection pressure but has evolved to mediate regulation.