

**P006** Roles of the ESCRT-machinery in retroviral budding and cytokinesis

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The Endosomal Sorting Complex Required for Transport (ESCRT) machinery is a multi-subunit protein complex that regulates cellular membrane fission events such as retroviral budding and formation of multivesicular bodies upon endosomes. Here, we identify a novel role for this machinery in the topologically equivalent process of cytokinesis. We show that suppression of ESCRT-components results in a failure of abscission, demonstrating an essential role these proteins in cytokinesis. Drawing parallels with retroviral recruitment of the ESCRT machinery, we show that the Flemming body component Cep55 (Centrosome Associated Protein, 55kDa) recruits this machinery to the midbody through direct interaction with two ESCRT-components, namely Tsg101 (Tumor-Susceptibility Gene 101) and Alix (Alg2 interacting protein-X). We map the binding sites for these proteins and, using siRNA-depletion and rescue experiments, we examine the mechanistic requirements for these proteins in cytokinesis. We show that Cep55/Tsg101/ESCRT-I and Cep55/Alix/ESCRT-III interactions are required for cytokinesis, demonstrating a key role for this machinery in abscission. Further, we show that disrupting Cep55/Alix/ESCRT-III interactions results in the formation of morphologically abnormal midbodies, recapitulating the effect of Cep55 depletion upon midbody morphology and suggesting that the ESCRT-machinery represents a major Flemming body component. We also map novel intra-ESCRT interactions and demonstrate differential requirements for this machinery in HIV-1 release and cytokinesis.