Reconstitution of vimentin intermediate filament dynamics *in vitro*

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Intermediate filaments (IFs) are an important component of the cytoskeleton in addition to actin and microtubules, involved in many cellular functions such as polarity, migration, division or maintenance of cell integrity. Vimentin is a widespread IF expressed mainly in mesenchymal cells. Hence it is used as a standard marker for epithelial-mesenchymal transition and epithelial cancers. In cells, vimentin network extends from the nucleus to the plasma membrane and constantly reorganize through constant severing and reannealing of filaments together with subunit exchange along the filaments. Despite direct observation in cells, the mechanisms involved vimentin dynamics are not fully understood. Here, we used *in vitro* reconstitution of vimentin from purified proteins to characterize their dynamical properties. First, we observed from assembly assays that vimentin filaments reached an equilibrium length at long timescales (>24h). This suggests that vimentin assembly reaches a steady-state resulting from an equilibrium between assembly and disassembly, which has always been neglected in previous studies. Then, upon reducing the concentration of pre-assembled filaments, we observed that filaments decreased both their length and mass per unit length upon time, indicating that filaments both break and lose mass through disassociation of subunits upon dilution. Finally, we showed that subunit exchange results from an equilibrium between subunit association and disassociation along the filaments which occur simultaneously with vimentin assembly. From our experimental results combined with numerical simulations, we propose that the continuous association and disassociation of vimentin subunits could randomly induce defects in filaments, that could lead to local severing. Our study provides new insights into the understanding of dynamics of vimentin filaments, of which reversible assembly and turnover take place without the addition of other proteins involved in disassembly.