1 Abstract

Wnt/β-catenin signalling plays a major role in almost all biological processes like embryogenesis, development and homeostasis. Therefore, countless studies over the past decades from multiple research fields unravelled the structure of β-catenin and its interaction partners as well as the kinetics of Wnt-dependent and -independent β-catenin activation. However, surprisingly, there is still a lack of knowledge about the spatio-temporal β-catenin activation, as the key player in Wnt/β-catenin signalling. The correlation of β-catenin structure and function with its activation in time and space will provide insight into the dynamic regulation of β-catenin at the single cell level.

One possible tool, an intramolecular biosensor monitoring the stability of β-catenin in single living cells, is presented in this thesis. The basic principle, used to measure the relative stability of the sensor molecule, is the difference in maturation kinetics of a FRET pair of two fluorophores. With this, the sensor molecule contains an intramolecular "concentration" and a "half-lifetime" marker.

In addition, genetic modifications of β-catenin yield a sensor (1) specific for monitoring only Wnt-dependent (canonical Wnt/β-catenin pathway) ß-Catenin activation, as well as (2) it does not report on β-catenin stability changes mediated via growth factor stimulation of RTKs and is (3) inert for downstream transcriptional activation. Therefore, the biosensor neither interferes with the degradation kinetics of endogenous β-catenin nor the downstream effectors or the induction of feedback loops. These modifications ensure, that fold change by stabilization upon Wnt ligand induction were recorded by the stability sensor.

Wnt stimulation induced stabilization of the sensor molecules within hours after application, measurable with FLIM and ratiometric widefield imaging. Comparison with available transcriptional reporter systems revealed a faster response time for the sensor molecules on single-cell level. Thus, the sensor, but not reporters with delayed response times, enables the detection of early Wnt-dependent signalling events as well as cell history and fate with live cell imaging.