4.5. Summary & Outlook:

The Ras-GEF hSOS1 catalyzes the nucleotide exchange of Ras. More than one decade ago it was found that prenylation of Ras is required for an efficient hSOS1 promoted nucleotide-exchange of Ras. Recent findings show that hSOS1 is regulated by a positive-feedback-activation and blocking this allosteric site manifests in an autoinhibition. None of the latter two findings were investigated in respect to prenylation of Ras.

Here, it was found that prenylation of allosteric bound Ras and prenylation of Ras bound to the active site are necessary and sufficient for releasing hSOS1 N-terminal autoinhibition at least partially and in vitro. More it was found that the PH-domain of hSOS1 senses the prenylation state of allosteric-bound Ras and allows even GDP-loaded Ras to stimulate hSOS1 basal activity. The mechanism underlying this sensing is currently elusive, since no direct interaction of allosteric Ras and the PH-domain could be observed. Products of the PI3-kinase (PIP₃), reported to bind to the PH-domain were shown to have no influence on releasing autoinhibition. More allosteric feedback-activation of hSOS1 is restricted to Ras itself as other Ras-GTPases could not compensate for allosteric bound Ras.

At least the prenylsensitivity of active site-bound Ras seems to be mediated indirectly by lipid-induced structural changes and not by a direct binding of the lipid-modified C-terminus to the catalytic fragment of hSOS1. An interesting observation is that GST-tagging of Ras changes Ras properties regarding the interaction with hSOS1 to higher nucleotide exchange of GST-Ras compared to non-GST-tagged Ras.

Initial nucleotide exchange-reactions performed at artificial membranes using TIRF-microscopy suggest that nucleotide-exchange is not significantly different from that in a membrane-free system.

Further research should focus on the mechanisms underlying the sensor-functions of the PH-domain regarding the assistance in binding of prenylated allosteric Ras. The recent development of fully processed K-Ras4B-proteins in our group by native chemical ligation allows the use of these proteins for stable membrane-binding at artificial negatively-charged membranes. This system can then be used to validate the initial findings of SOS⁷⁵⁶-promoted nucleotide exchange at the membrane.