

MS04 Structure in Cancer Biology

MS4-03

Equilibria between conformational states of the Ras oncogene protein revealed by high-pressure crystallography
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Abstract

The small GTPase Ras protein, involved in central regulatory processes such as cell differentiation, proliferation and apoptosis, possesses multiple conformational states and acts as a molecular switch between active GTP-bound, and inactive GDP-bound states, controlling essential signal transduction pathways. Ras is anchored to the membrane via its C-terminal domain and activated through cell surface receptors. An allosteric network of interactions between the effector binding regions and the membrane interacting regions is involved in Ras cycling. The different conformational states which coexist simultaneously in solution with low occupancies possess higher Gibbs free energy than the ground state. Equilibria between these states can be shifted by applying high hydrostatic pressure favoring conformations with lower molar partial volumes and has been previously analyzed by high-pressure NMR spectroscopy.

High-pressure macromolecular crystallography is a powerful tool to characterized at the molecular level the different allosteric states involved in Ras cycling. High resolution structures of Ras(wt).Mg²⁺.GppNHp and Ras(D33K).Mg²⁺.GppNHp at pressures up to 900 MPa give insight to per-residue descriptions of the structural plasticity involved in allosteric equilibria between conformers. A transition above 300 MPa in the crystal leads to more stable conformers. The different segments of Ras protein which remains in the ground-state conformation or undergo structural changes, adopting excited-energy conformations corresponding to transient intermediate states, have been mapped out at atomic resolution. Such in-crystallo phase transitions induced by pressure opens the possibility to finely explore the structural determinants related to switching between Ras allosteric sub-states without any mutations nor exogenous partners.

References

Girard et al., Chem. Sci., 2022, 13:2001-2010

