Methods

Protein purification and crystallization

We prepared RanGAP from *Schizosaccharomyces pombe* and human Ran as described. Human RanBP1 was expressed in BL21 *Escherichia coli* as a glutathione *S*-transferase fusion protein and subsequently purified with Glutathione–Sepharose 4 Fast Flow (Amersham Pharmacia). We loaded RanBP1 onto an affinity column and added Ran–GppNHp in twofold excess afterwards. The binary complex was eluted after a 12-h incubation with thrombin protease. We then added RanGAP in excess and purified the ternary complex by size-exclusion chromatography in buffer (20 mM Tris-HCl pH 7.5, 2 mM $MgCl_2$, 2 mM dithioerythritol (DTE)). Crystals were grown by hanging drop from 18.5–20.5% PEG4000, 100 mM potassium acetate, 100 mM Tris-HCl pH 7.5 for the GppNHp complex. After 3–4 d at 20 °C, thin needle protein crystals appeared ($500 \times 45 \times 45\,\mu m^3$).

For the transition-state analogue complex, RanBP1, Ran–GDP and RanGAP were mixed in a buffer containing 2 mM Al^{3+} and 30 mM NaF. Subsequently, the ternary complex was purified by size-exclusion chromatography in 20 mM Tris-HCl pH 7.5, 2 mM $MgCl_2$, 2 mM DTE containing additional 30 mM NaF. Crystals were grown from 18.5–20.5% PEG4000, 100 mM Tris-HCl pH 7.5 at 20 °C.

Data collection and structure determination

The crystals, with space group *P*1 and unit-cell dimensions of $a = 101.6$ Å, $b = 103.1$ Å, $c = 120.2$ Å, $\alpha = 71.6$, $\beta = 80.6$, $\gamma = 67.8$, were very sensitive to radiation damage. Data sets were obtained by translating a single crystal needle and successively exposing small sections to the beam. Data were collected on MAR image plate detectors at the beam lines ID13 and ID14-1 (for AlF_x) at the European Synchrotron Radiation Facility (ESRF) and were processed with DENZO[27]. We solved the structure of the complex by molecular replacement using AMORE[28] with RanGAP[7] as a model (correlation coefficient 0.41, *R*-factor 0.47). The initial electron density was improved greatly by four-fold averaging using the non-crystallographic symmetry (NCS) in the unit cell. After inclusion of Ran and RanGAP the DM[28] correlation coefficient for the RanBP1 electron density was 0.77. The model of the ternary complex was built using the program O[29] and refined with CNS applying NCS restraints[30]. The final model comprises residues 2–345 of RanGAP, 8–213 of Ran and 22–167 of RanBP1.

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Competing interests statement

The authors declare that they have no competing financial interests.

Correspondence and requests for materials should be addressed to A.W. (e-mail: alfred.wittinghofer@mpi-dortmund.mpg.de). Coordinates have been deposited with the Protein Data Bank (accession numbers 1K5D and 1K5G).

erratum

Formation of coastline features by large-scale instabilities induced by high-angle waves

Andrew Ashton, A. Brad Murray & Olivier Arnoult

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In this Letter, the last name of the third author, Olivier Arnoult, was misspelled as 'Arnault'. □