Conserved forkhead dimerization motif controls DNA replication timing and spatial organization of chromosomes in S. cerevisiae

Ostrow, A.Z.; Kalhor, R.; Gan, Y.; Villwock, S.K.; Linke, C.; Barberis, M.; Chen, L.; Aparicio, O.M.

Published in:
Proceedings of the National Academy of Sciences of the United States of America

DOI:
10.1073/pnas.1612422114

Link to publication

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Fig. S1. Fkh1 and Fkh2 share key residues and predicted structure with human FoxP family proteins. (A) Multiple sequence alignment of additional human and fission yeast (sp) forkhead domains. Amino acid numbers are based on Fkh1, and specific residues discussed in the text are highlighted. (B) Amino acid identities and similarities in Forkhead proteins across species. (C) Modeled forkhead domain structures of Fkh1 (orange) and Fkh2 (pink) overlaid on the solved structure of FoxP3 domain-swapped dimer (red).
Fig. S2. Fkh1 dimerizes in vitro. (A) Coomassie-stained SDS/PAGE of E. coli-expressed and purified His-Fkh1-FD (lane 1). (B) Pull-down assay with Fkh1-Myc or Fkh1-dsm-Myc isolated from yeast whole-cell extracts (strains ZOy46 and ZOy50, respectively) and immobilized on beads, incubated with E. coli-expressed and purified His-Fkh1-FD. Bound material was analyzed by immunoblotting with a polyclonal anti-Fkh1 antibody. (C) Quantification of results of three experiments as in B showing the average (±SD) ratio of His-Fkh1-FD pulled down by Fkh1-dsm-Myc versus Fkh1-Myc. (D) Bacterially produced GST, GST-Fkh1, and GST-Fkh1-dsm were immobilized on Glutathione Sepharose beads and incubated with yeast lysate containing Fkh1-Myc (lanes 1–4) or Fkh1-dsm-Myc (lanes 5–8). Pull-downs (Upper) and Input (Lower) were analyzed by immunoblot analysis using anti-Myc antibody. (E) Quantification of data in D showing the ratio of Fkh1-dsm-Myc pulled down by GST-Fkh1-dsm to Fkh1-Myc pulled-down by GST-Fkh1.

Fig. S3. Fkh1 interaction with ORC is unaffected by dsm mutation. Immunoprecipitation of Fkh1-Myc and Fkh1-dsm-Myc from cell extracts of strains ZOy46 and ZOy50, respectively, was analyzed by immunoblotting with anti-ORC polyclonal antibody.
Fig. S4. ARS305 4C interactions along chromosome XII. Strains ZOy20 (fkh2Δ) and ZOy10 (fkh1-dsm fkh2Δ) were synchronized in G1 phase and subjected to 4C analysis with ARS305 as bait; only the FKH1 genotype is indicated on the images. Spheres below the chromosomal plots denote replication origins, with red indicating Fkh-activated and green indicating Fkh-repressed.