The double-stranded DNA-binding proteins TEBP-1 and TEBP-2 form a telomeric complex with POT-1

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Telomeres are bound by dedicated proteins, which protect them from DNA damage and regulate telomere length homeostasis. In the nematode Caenorhabditis elegans, a comprehensive understanding of the proteins interacting with the telomere sequence is lacking. Here, we harnessed a quantitative proteomics approach to identify TEBP-1 and TEBP-2, two paralogs expressed in the germline and embryogenesis that associate to telomeres in vitro and in vivo. tebp-1 and tebp-2 mutants display strikingly distinct phenotypes: tebp-1 mutants have longer telomeres than wild-type animals, while tebp-2 mutants display shorter telomeres and a Mortal Germline. Notably, tebp-1;tebp-2 double mutant animals have synthetic sterility, with germlines showing signs of severe mitotic and meiotic arrest. Furthermore, we show that POT-1 forms a telomeric complex with TEBP-1 and TEBP-2, which bridges TEBP-1/-2 with POT-2/MRT-1. These results provide insights into the composition and organization of a telomeric protein complex in C. elegans.
Most telomeres in linear eukaryotic chromosomes end in tandem repeat DNA sequences. Telomeres solve two major challenges of chromosome linearity: the end-protection problem and the end-replication problem\(^1\)\(^,\)\(^2\). The end-protection problem originates from the structural similarity between telomeres and DNA double-strand breaks, which can lead to recognition of the telomere by the DNA damage surveillance machinery\(^3\). When telomeres are falsely recognized as DNA damage, they are processed by the non-homologous end joining or homologous recombination pathways, leading to genome instability\(^4\)\(^,\)\(^5\). The end-replication problem arises from the difficulties encountered by the DNA replication machinery to extend the extremities of linear chromosomes, which results in telomere shortening with every cell division\(^6\)\(^,\)\(^7\). When a subset of telomeres shorten beyond a critical point, cellular senescence or apoptosis is triggered\(^8\)\(^,\)\(^9\)\(^,\)\(^10\).

Specialized proteins have evolved to deal with the complications arising from telomeres, which in vertebrates are composed of double-stranded (ds) (TTAGGG)\(_n\) repeats ending in a single-stranded (ss) 3' overhang\(^11\). In mammals, a telomere-interacting complex of six proteins termed shelterin constitutively binds to telomeres in mitotic cells\(^12\). This complex consists of the ds telomere binders TRF1 and TRF2, the TRF2-interacting protein RAP1, the ss binding protein POT1 and its direct interactor TPP1, as well as the bridging protein TIN2. Altogether, the proteins of this complex shield telomeres from a DNA damage response by inhibiting aberrant DNA damage signaling\(^1\). In addition, shelterin components are required for the recruitment of the telomerase enzyme, which adds de novo repeats to the telomeric ends, allowing maintenance of telomere length in dividing cells\(^8\). Telomerase is a ribonucleoprotein, comprised of a catalytic reverse-transcriptase protein component and an RNA moiety. Besides the core shelterin complex, additional proteins have been described to interact with telomeres and assist in the maintenance of telomere length, e.g., HMBOX1 (also known as HOT1), ZBTB48 (also known as TZAP), NR2C2, and ZNF827\(^13\)\(^,\)\(^14\)\(^,\)\(^15\).

In Schizosaccharomyces pombe, a shelterin-like complex harboring orthologs of the human shelterin complex was described\(^16\)\(^,\)\(^17\)\(^,\)\(^18\)\(^,\)\(^19\)\(^,\)\(^20\). TAZ1 and POT1 bind to ds and ss telomeric DNA similar to their human counterparts TRF1/TRF2 and POT1, respectively. In turn, Saccharomyces cerevisiae has distinct complexes binding to the ds and ss telomeres\(^21\)\(^,\)\(^22\)\(^,\)\(^23\)\(^,\)\(^24\)\(^,\)\(^25\)\(^,\)\(^26\). The S. cerevisiae ortholog of the TRF2-interacting protein RAP1 binds ds telomeric DNA through two domains structurally related to Myb domains\(^27\). The ss overhang is not bound by a POT1 homolog but rather by the CST complex\(^22\)\(^,\)\(^23\)\(^,\)\(^25\). Overall, this indicates that different telomere-binding complexes have evolved across species to alleviate the challenges of linear chromosome ends, based on variations of recurring DNA-binding modules.

The nematode Caenorhabditis elegans has been employed in many seminal discoveries in molecular biology, genetics, and development\(^38\). Its telomeres have a repeat sequence similar to vertebrate telomeres, consisting of (TTAGGC)\(_n\). Moreover, C. elegans telomeres have a length of about 2–9 kbp\(^29\)\(^,\)\(^30\), and it has been proposed that its telomeric structures have both 5' and 3' ss overhangs, each recognized by dedicated ss telomere-binding proteins\(^31\). Telomere maintenance in this nematode is carried out by the catalytic subunit of telomerase TRT-1\(^,\)\(^32\). The RNA component of C. elegans telomerase has not been identified thus far. Telomeres can be maintained by additional mechanisms, since C. elegans can survive without a functioning telomerase pathway by employing alternative lengthening of telomere (ALT)-like mechanisms, creating more heterogeneous telomere lengths\(^33\)\(^,\)\(^34\)\(^,\)\(^35\).

In C. elegans, four proteins with domains structurally similar to the DNA-binding domain of human POT1 were identified. Three of those proteins, namely POT-1 (also known as CeOB2), POT-2 (also known as CeOB1), and MRT-1, were confirmed to bind to the ss telomeric overhangs\(^33\)\(^,\)\(^36\). Mutants for these factors show telomere length maintenance defects. Depletion of POT-1 and POT-2 leads to telomere elongation\(^33\)\(^,\)\(^36\)\(^,\)\(^37\), whereas depletion of MRT-1 results in progressive telomere shortening over several generations\(^38\). Concomitant to telomere shortening, mrt-1, mrt-2, and trt-1 mutant animals share a Mortal Germline (Mrt) phenotype, characterized by a gradual decrease in fertility across generations, until animals become sterile\(^30\)\(^,\)\(^32\)\(^,\)\(^38\). MRT-1 was proposed to be in a pathway for facilitation of telomere elongation together with the DNA damage checkpoint protein MRT-2, and telomerase TRT-1\(^,\)\(^39\). Despite the identification of these different telomere-associated proteins, no telomere-binding complex has been described in C. elegans yet.

In this work, we performed a quantitative proteomics screen to identify novel telomere-binding proteins in C. elegans. We report the identification and characterization of R06A4.2 and T12E12.3, two previously uncharacterized paralog genes, which we named telomere-binding proteins 1 and 2 (tebp-1 and tebp-2), respectively. TEBP-1 and TEBP-2 bind to the ds telomeric sequence in vitro with nanomolar affinity and co-localize with POT-1, a known telomere binder, in vivo. tebp-1 and tebp-2 mutants have contrasting effects on telomere length: while tebp-1 mutants display elongated telomeres, tebp-2 mutants have shortened telomeres. In addition, TEBP-1 and TEBP-2 have important roles in fertility, as tebp-1; tebp-2 double mutants are synthetic sterile.

Results

TEBP-1 (R06A4.2) and TEBP-2 (T12E12.3) are double-stranded telomere-binding proteins in Caenorhabditis elegans. To identify proteins that bind to the C. elegans telomeric sequence, we employed a DNA pulldown assay (Supplementary Fig. 1a, b) previously used to successfully identify telomeric proteins in other species\(^15\)\(^,\)\(^16\)\(^,\)\(^39\). We incubated concatenated, biotinylated DNA oligonucleotides consisting of either the telomeric sequence of C. elegans (TTAGGC\(_n\)), or a control sequence (AGGTCA\(_n\)), with nuclear-enriched extracts of gravid adult worms. The experiment was performed twice using two different quantitative proteomics approaches: label-free quantitation (LFQ)\(^41\) and reductive dimethyl labeling (DML)\(^42\), which yielded 12 and 8 proteins enriched in telomeric sequence pulldowns, respectively, with an overlap of 8 proteins (Fig. 1a, b and Supplementary Fig. 1a, b). Among these eight proteins, we found the already known ss telomere binders POT-1, POT-2, and MRT-1\(^31\)\(^,\)\(^33\)\(^,\)\(^37\)\(^,\)\(^38\), as well as the CKU-70/CKU-80 heterodimer\(^43\), and three additional proteins: R06A4.2, T12E12.3, and DVE-1.

R06A4.2 and T12E12.3 were of particular interest, as they share 74.3% DNA coding sequence identity and 65.4% amino acid sequence identity (Supplementary Fig. 1c), suggesting that R06A4.2 and T12E12.3 are paralogs. While R06A4.2 and T12E12.3 lack any annotated protein domain, using HHpred v3.2.0\(^44\), we could determine that the N-terminal region of both R06A4.2 and T12E12.3 are paralogs. While R06A4.2 and T12E12.3 lack any annotated protein domain, using HHpred v3.2.0\(^44\), we could determine that the N-terminal region of both proteins shows similarity to the homeodomains of human and yeast RAP1 (Supplementary Fig. 1d, e and Supplementary Data file 1). RAP1 is a direct ds telomere binder in budding yeast\(^21\)\(^,\)\(^45\), and a member of the mammalian shelterin complex through interaction with TRF2\(^46\).

We validated binding of R06A4.2 and T12E12.3 to telomeric DNA by performing DNA pulldowns with His-tagged recombinant proteins (Fig. 1c). Using CRISPR-Cas9 genome editing, we
inserted a gfp and a 3xflag sequence directly upstream of the endogenous stop codon of T12E12.3 and R06A4.2, respectively (Supplementary Fig. 1d, e). Using these strains, we could show that the endogenously tagged versions of R06A4.2 and T12E12.3 also bind to the C. elegans telomere sequence (Fig. 1d).

Owing to the preparation strategy, our concatenated DNA probes contained both ds and ss DNA, which precludes any conclusions about whether R06A4.2 and T12E12.3 bind ss or ds telomeric DNA. We thus performed additional DNA pulldowns with ss and ds probes specifically designed with five repeats (TTAGGC)₅. Both proteins were found to exclusively bind to the ds telomeric repeats, establishing R06A4.2 and T12E12.3 as ds telomere binders (Fig. 1e, f). To confirm and quantify the interaction of R06A4.2 and T12E12.3 with ds telomeric DNA, we

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performed fluorescence polarization with purified, recombinant proteins and FITC-labeled oligonucleotides. Both T12E12.3 and R06A4.2 displayed affinity for the ds telomeric repeat sequence in the nanomolar range ($K_d = 128.7$ nM for R06A4.2 and $K_d = 37.84$ nM for T12E12.3, Fig. 1g, h). Both T12E12.3 and R06A4.2 showed highest affinity for the 2.5x telomeric repeat, when incubated with a 2.5x, 2.0x, 1.5x T-rich, and 1.5x G-rich telomeric repeat sequences (Supplementary Fig. S2a–c).

In conclusion, we demonstrate that R06A4.2 and T12E12.3, two proteins with highly similar sequence, bind directly and with high affinity to the *C. elegans* ds telomeric DNA sequence in vitro. Thus, we decided to name R06A4.2 as Telomere-Binding Protein-1 (TEBP-1) and T12E12.3 as Telomere-Binding Protein-2 (TEBP-2).

TEBP-1 and TEBP-2 localize to telomeress in proliferating cells in vivo. To explore the expression pattern of *tebp-1* and *tebp-2* throughout animal development, we used a recently published mRNA-seq dataset. Both genes show the highest expression in embryos, very low abundance during the L1-L3 larval stages, and an increase in expression in L4 larvae and young adults (YAs, Supplementary Fig. 3a–c). The observed increase in *tebp-1* and *tebp-2* mRNA expression from the L4 to YA stages coincides with the increased progression of germline development, which may hint to a higher expression level during gametogenesis. Indeed, using available gonad-specific RNA-seq datasets, we confirmed that *tebp-1* and *tebp-2* are expressed in spermatogenic and oogenic gonads (Supplementary Fig. 3d). Similar developmental mRNA expression patterns were also found for the known ss telomere binders *pot-1*, *pot-2*, and *mrt-1* (Supplementary Fig. 3a, d). To study the expression at the protein level, we crossed our endogenously tagged strains to generate a *tebp-1::3xflag; tebp-2::gfp* strain to monitor protein abundance simultaneously by western blot. The protein expression patterns of TEBP-1 and TEBP-2 are highly similar to the RNA-seq data, with highest detected expression in embryos, a drop during the larval stages L1-L4, ultimately followed by an increase in YA (Fig. 2a).

To study TEBP-1 and TEBP-2 localization in vivo, we focused on embryos and on the germline of adult animals. In these two actively dividing tissues, TEBP-1 and TEBP-2 protein expression is high and condensed chromosomes facilitate visualization of telomeric co-localization. In addition to the *tebp-2::gfp* strain used above, we also generated an endogenously tagged *tebp-1::gfp* allele, using CRISPR-Cas9 genome editing (Supplementary Fig. 1d). To check for telomeric localization in vivo, we crossed *tebp-1:: GFP* and *tebp-2::GFP* each with a germline-specific *pot-1::mCherry* single-copy transgene, and imaged the dual-fluorescent animals. TEBP-1::GFP and TEBP-2::GFP co-localize with *PO T-1::mCherry* inside the nuclei of oocytes and embryos (Fig. 2b–e). Confocal microscopy of TEBP-1::GFP in combination with *POT-1::mCherry* was challenging likely due to bleaching of TEBP-1::GFP. Co-localization of TEBP-2::GFP and *POT-1::mCherry* was also observed in the mitotic region of the germline and in mature sperm (Fig. 2d). These results clearly establish that TEBP-1 and TEBP-2 co-localize with a known telomeric binder in vivo in proliferating tissues, indicating that their ability to bind ds telomeric DNA in vitro may have functional relevance.

TEBP-1 and TEBP-2 have opposing telomere length phenotypes. As TEBP-1 and TEBP-2 localize to telomeress, we sought to address whether these proteins regulate telomere length, as is the case for the known ss telomere-binding proteins *PO T-1*, *PO T-2*, and *MRT-1*. Using CRISPR-Cas9 genome editing, we generated *tebp-1* and *tebp-2* deletion mutants encoding truncated transcripts with premature stop codons (Supplementary Fig. 1d–g and Supplementary Fig. 4a, b). *tebp-1* and *tebp-2* mutants are viable and show no immediate, obvious morphological or behavioral defects. We analyzed telomere length in the mutants after propagation for more than 100 generations, sufficient to establish a “steady-state” telomere length phenotype, by carrying out a telomere Southern blot on mixed-stage animals. Interestingly, while *tebp-1*(sfl333) shows an elongated telomere phenotype comparable to the *pot-2*(tm1400) mutant, *tebp-2*(sfl313) shows a shortened telomere phenotype (Fig. 3a), similar to *mrt-1* mutants. In addition, we performed quantitative fluorescence in situ hybridization (qFISH) in dissected adult germlines, which confirmed our initial observation that *tebp-1* and *tebp-2* mutants display longer or shorter telomeress than wild-type, respectively (Fig. 3b–f). Furthermore, we also measured telomere length in embryos by qFISH. Like in the germline, the telomeres of *tebp-1* mutant embryos are elongated, while the telomeres of *tebp-2* embryos are shortened (Supplementary Fig. 4c–g).

In summary, *tebp-1* and *tebp-2* mutants display opposing regulatory effects on telomere length. These experiments suggest that the TEBP-1 protein counteracts telomere elongation...
Fig. 2 TEBP-1 and TEBP-2 are expressed throughout *C. elegans* development and localize to telomeres in vivo. a Western blot of TEBP-1::3xFLAG and TEBP-2::GFP expression in different developmental stages of *C. elegans*. Thirty-five micrograms of extract from either N2 or a double transgenic line carrying TEBP-1::3xFLAG and TEBP-2::GFP were used. Actin was used as loading control. kDa: kilodalton. Uncropped blot in Source Data. N = 1. b, c Maximum intensity projections of representative confocal z-stacks of an embryo (b), or oocytes (c) expressing endogenously tagged TEBP-1::GFP and transgenic POT-1::mCherry. Scale bars, 10 μm. d, e Maximum intensity projections of representative confocal z-stacks of an adult animal (d), or embryo (e) expressing both endogenously tagged TEBP-2::GFP and transgenic POT-1::mCherry. Insets show nuclear co-localization in meiotic germ cell nuclei (I), an oocyte (II), spermatozoa (III), and embryonic cells (IV). Scale bars, 20 μm (overview) and 4 μm (insets). All microscopy images were deconvoluted using Huygens remote manager. Representative images from two individual animals per strain, N = 2 biologically independent experiments with similar results.
independently of telomerase, while TEBP-2 promotes telomere lengthening.

Simultaneous lack of TEBP-1 and TEBP-2 leads to synthetic sterility. To better understand how *tebp-1* and *tebp-2* mutants distinctly affect telomere length, we intended to measure telomere length in *tebp-1*; *tebp-2* double mutants. Surprisingly, when we crossed our single mutants, we could not establish a double homozygous *tebp-1*; *tebp-2* mutant strain. In fact, *tebp-1*; *tebp-2* double mutants displayed highly penetrant synthetic sterility (Fig. 4a). Repeating the cross with another *tebp-1* mutant allele (*xf134*), as well as the reciprocal cross, yielded the same synthetic sterility (Fig. 4b and Supplementary Fig. 5a). Only about 14–38% of F2 or F3 *tebp-1*; *tebp-2* animals did not have synthetic sterility (Fig. 4a, b). These “synthetic sterility escapers” were subfertile, siring less than 60 offspring. Importantly, a *tebp-2::gfp* single-copy transgene fully rescued the appearance of sterility, demonstrating that the C-terminal tag does not disrupt TEBP-2 function (Supplementary Fig. 5a). When we combined *tebp-1* mutant animals with *mrt-1*, *trt-1*, or *pot-2* mutations, or *tebp-2* mutant animals with *trt-1* or *pot-2*, the double mutant offspring was fertile (Supplementary Fig. 5a). These results demonstrate that the synthetic sterility is specific to *tebp-1*; *tebp-2* double mutants, and is not a consequence of crossing shorter telomere mutants with longer telomere mutants. We further quantified the synthetic sterility on brood size by picking L2-L3 progeny of *tebp-2*; *tebp-1*+/− mutants, blind to genotype and germline health, rearing those
animals at 20°C or 25°C, later counting their brood sizes, and genotyping each animal (Fig. 4c–e). This revealed that the immediate synthetic sterility phenotype is not dependent on temperature, as the reduction of progeny numbers was apparent at both 20 and 25°C.

Morphologically, tebp-1; tebp-2 double mutants displayed a degenerated germline. To visualize this phenotype, we created tebp-1 and tebp-2 strains in combination with an endogenously tagged pgl-1::mTagRfp-T allele49,50, which we used as a germ cell reporter. PGL-1 is expressed in P-granules, perinuclear granules most important for germline development and gene regulation51,52. As depicted in Fig. 4f, we repeated the tebp-1 x tebp-2 cross with pgl-1::mTagRfp-T in the background, isolated cross progeny of the indicated genotypes, reared these animals to adulthood, scored them into three categories of germline morphology, and genotyped them afterwards. The categories can be described as follows: category 1 animals displayed a wild-type or near wild-type morphology (Fig. 4g, upper panels),

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| Category | Description                  | Genotype                                      |
|----------|------------------------------|-----------------------------------------------|
| Category 1 | Sub-fertile                  | tebp-1(xf133) +/-; tebp-2(xf131) +/-; pgl-1::mTagRfp-T |
| Category 2 | Male                         | tebp-1(xf133) +/-; tebp-2(xf131) +/-; pgl-1::mTagRfp-T |
| Category 3 | Sterile                      | tebp-1(xf133) +/-; tebp-2(xf131) +/-; pgl-1::mTagRfp-T |

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Fraction fertile over generations for various genotypes (Fig. 4i).
category 2 animals displayed one atrophied gonad arm (Fig. 4g, middle panels), and category 3 animals had both gonad arms atrophied (Fig. 4g, lower panels). Besides Fig. 4g, representative animals for categories 2 and 3 are shown in Supplementary Fig. 5b. More than 85% of tebp-1; tebp-2; pgl-1::mTagRfp-T worms had a category 3 germline, while the remainder had only one gonad arm atrophied (Fig. 4h). Atrophied gonads generally showed under-proliferation of the germ cell nuclei of the mitotic zone and rare entry into meiosis, suggesting severe defects in cell division (Fig. 4g and Supplementary Fig. 5b). In addition, almost 15% (17/114 animals) of the progeny of tebp-1; tebp-2; pgl-1::mTagRfp-T synthetic sterility escapers were males, indicative of a high incidence of males (Him) phenotype. The synthetic sterility escaper progenies of previous crosses were also Him, at least in some cases (see F3 escaper progeny in Fig. 4b). Lastly, approximately 8% (8/97) of hermaphrodite tebp-1; tebp-2; pgl-1::mTagRfp-T escaper progeny had growth defects: while some reached adulthood but remained smaller than wild-type, others arrested prior to adulthood (Supplementary Fig. 5c).

Overall, these data show that the lack of functional TEBP-1 and TEBP-2 leads to severe germline defects that impede germline development.

TEBP-2 is required for transgenerational fertility. Despite the synthetic sterility of the double mutants, tebp-1 and tebp-2 single mutants did not have a baseline reduction in fertility when grown at 20 and 25 °C (Supplementary Fig. 5d, e). Nevertheless, mutants of telomere regulators, like trt-1 and mrt-1, exhibit a Mrt phenotype, characterized by progressive loss of fertility across many generations. \( ^{32,38} \) We thus conducted a Mortal Germline assay at 25 °C using late generation mutants, and found that tebp-1 and tebp-2 mutants displayed opposing phenotypes in line with their differing effects on telomere length. While tebp-1(sf133) remained fertile across generations, like wild-type, tebp-2(sf131) showed a Mrt phenotype (Fig. 4i), the onset of which is delayed compared to mrt-1(tm1354) and trt-1(ok410), indicating a slower deterioration of germline health over generations. These results show that TEBP-2 is required to maintain germline homeostasis transgenerationally, while TEBP-1 is not.

TEBP-1 and TEBP-2 are part of a telomeric complex in *C. elegans*. Our initial mass spectrometry approach allowed us to identify proteins associated with the telomeres of *C. elegans*. However, it remains unknown if these factors interact and whether they are part of a telomere-binding complex. To address this, we performed size-exclusion chromatography with embryonic extracts from a strain expressing TEBP-1::3xFLAG; TEBP-2::GFP. Western blot analysis of the eluted fractions shows that TEBP-1 and TEBP-2 have very similar elution patterns with one peak ranging from 450 kDa to 1.5 MDa, with a maximum at 1.1 MDa (Fig. 5a and Supplementary Fig. 6a). Next, we reasoned that the elution peak would shift if telomeric DNA is enzymatically degraded. To test this, embryonic extracts were treated with *Serratia marcescens* nucleases (Sm nucleases), a non-sequence-specific nuclease, prior to size-exclusion chromatography, but we did not observe a strong shift (Fig. 5b). While we cannot fully exclude the possibility that telomeric DNA was inaccessible to Sm nuclease digestion, the results suggest that TEBP-1 and TEBP-2 are part of a telomeric complex.

To identify proteins interacting with TEBP-1 and TEBP-2, we performed immunoprecipitation (IP) followed by quantitative mass spectrometry (qMS) in embryos (Fig. 5c, d) and YAs (Supplementary Fig. 6b, c). Notably, IP-qMS of TEBP-1 and TEBP-2 baits enriched for MRT-1, POT-1, and POT-2, the three known ss telomere-binding proteins in *C. elegans*. In some cases, (Fig. 5d and Supplementary Fig. 6b) it was difficult to unambiguously assign unique peptides to TEBP-1::3xFLAG and TEBP-2::GFP in our qMS analysis, given their high protein sequence identity (65.4%). However, we confirmed by co-IP experiments that TEBP-1 and TEBP-2 reciprocally interact in embryos and YA (Fig. 5e, f and Supplementary Fig. 6d). Moreover, TEBP-1 and TEBP-2 remain associated with MRT-1, POT-1, and POT-2 even after treatment with Sm nucleases (Supplementary Fig. 6e, f).

**POT-1 is required to bridge the double-stranded and the single-stranded telomere.** To reveal the architecture of the telomeric complex, we sought to identify direct interactions amongst TEBP-1, TEBP-2, POT-1, POT-2, and MRT-1, using a yeast two-hybrid (Y2H) screen. While TEBP-2 fused to the DNA-binding domain of Gal4 unfortunately self-activated the reporter (Supplementary Fig. 6g), we could identify direct interactions of POT-1 with TEBP-1 and TEBP-2 (Fig. 6a and Supplementary Fig. 6g). Furthermore, in accordance with IP-qMS and co-IP experiments (Fig. 5e, f and Supplementary Fig. 6d), we confirmed interaction between TEBP-1 and TEBP-2 in the Y2H experiment (Fig. 6a and Supplementary Fig. 6g). These results are consistent with a scenario where TEBP-1 and TEBP-2 interact directly with each other and with POT-1.

The observed direct interactions suggest that POT-1 may be a critical link between the ds and the ss telomeric region. To test this idea, we performed IP-qMS of TEBP-1 and TEBP-2, in
Young adults

Y2H experiments using the fragments shown in Fig. 6d, indicate that the C-terminal tails of TEBP-1 and TEBP-2 (f7) interact with the OB-fold of POT-1 (Fig. 6f, g). Additional Y2H assays demonstrate that TEBP-1 and TEBP-2 interact with each other via their respective f1 fragments, encompassing their first predicted homeo-/myb-domains (Fig. 6h and Supplementary Fig. 6i).

Altogether, our data strongly indicate that TEBP-1 and TEBP-2 are integral parts of a telomeric complex, or complexes, which also include the known ss telomere binders POT-1, POT-2, and MRT-1. We propose a simple working model where TEBP-1 and TEBP-2 also include the known ss telomere binders POT-1, POT-2, and MRT-1. We propose a simple working model where TEBP-1 and TEBP-2 bind to the ds telomere via their third predicted homeo-/myb-domains, have opposed effects on telomere dynamics, and may link the ds binders to the ss telomere, thereby bringing TEBP-1 and TEBP-2 in close proximity of POT-2 and MRT-1 (Fig. 6i).
Conservation of *tebp* genes in the *Caenorhabditis* genus. To infer the evolutionary history of *tebp*-1 and *tebp*-2 genes, we identified protein-coding orthologs by reciprocal BLASTP analysis in the searchable genomes in Wormbase and Wormbase ParaSite databases. Then, we performed a multiple sequence alignment with the ortholog protein sequences, and used it to build a phylogenetic tree (Fig. 7a and Supplementary Data file 2). Our findings suggest that *tebp* orthologs are present only in the *Caenorhabditis* genus, mostly in the *Elegans* supergroup (which includes the *Elegans* and *Japonica* groups). A distinct number of protein-coding *tebp* genes was identified per species: *C. briggsae*, *C. nigoni*, *C. sinica*, and *C. japonica* have one *tebp* ortholog; *C. elegans*, *C. inopinata*, *C. remani*, *C. bremeri*, *C. tropicalis*, and *C. angaria* have two *tebp* orthologs; and *C. latens* has three *tebp*
Fig. 6 POT-1 links the ds telomere binders to the ss telomere. a Y2H assay with full length TEBP-1, TEBP-2, and POT-1 fusions to the activation or DNA-binding domains of Gal4. Growth on TRP LEU HIS plates demonstrates interaction. Growth on high stringency TRP LEU HIS ADE medium suggests strong interaction. TRP: lacking tryptophan, LEU: lacking leucine, HIS: lacking histidine, ADE: lacking adenine. b, c Volcano plots showing quantitative proteomic analysis of either TEBP-1:3xFLAG (b) or TEBP-2:GFP (c) IPs in embryos. IPs were performed in quadruplicates. Enriched proteins (threshold: 2-fold, p-value < 0.05) are shown as black dots, enriched proteins of interest are highlighted with red or orange dots, and annotated. Background proteins are depicted as gray dots and the respective bait protein annotated in red. d Scheme for the cloning of different fragments of TEBP-1, TEBP-2 and POT-1 for IP experiments and Y2H. TEBP-1 and TEBP-2 were divided into five fragments (f1–f5) of approx. 30 kDa, as well as two additional fragments covering the N-terminus including the predicted DNA-binding domains (f6) and the C-terminus (f7). POT-1 was divided into three fragments of around 15 kDa (f1–f3). e DNA pulldowns as in Fig. 1c with recombinantly expressed and N-terminally His-MBP-tagged fragments f1, f3, and f5 of TEBP-1 and TEBP-2, as well as the full length proteins with the same tags. The western blot was probed with α-His antibody and the signals detected by chemiluminescence. f1–f5: fragments of respective protein, full: full length respective protein, kDa: kilodalton, MBP: maltose-binding protein. N = 2 independent experiments with similar results. f Y2H assay like in (a) but with TEBP-1 and POT-1 full length proteins (f1), as well as N- and C-terminal fragments (f6 and f7 for TEBP-1, or f1 and f3 for POT-1, respectively) fused to the activation or DNA-binding domains of Gal4. Growth determined on the same medium as in a, g Y2H assay as in (f) but with TEBP-2 and POT-1 constructs. h Y2H assay as in (f) but with all fragments of TEBP-1 including the full length protein fused to the Gal4 DNA-binding domains, as well as all fragments of TEBP-2 including the full length protein fused to the Gal4 activation domain. f1–f7: fragments of respective protein, ctrl: control/empty plasmid, ff: full length protein. i Proposed working model for the interactions between telomere-binding proteins and telomere repeats in C. elegans. TEBP-1 and TEBP-2 fragments 3 (f3), containing a predicted DNA-binding domain, bind to ds telomere repeats and have opposing effects on telomere elongation. Both proteins interact with each other via their N-terminal fragments (f1). TEBP-1, TEBP-2 and POT-1 interact directly via the C-terminal fragment (f7) of TEBP-1/TEBP-2 and the N-terminal fragment (f1) of POT-1. As a result of this interaction, the ss telomere comes in closer contact to the ds telomere. Our current data does not support direct interactions between POT-1, POT-2, and MRT-1, but these factors may interact in the presence of telomeric DNA.

Robust identification of telomere-associated proteins in C. elegans. Three lines of evidence demonstrate the validity and robustness of our screen. First, attesting for its technical reproducibility, the two qMS detection strategies employed shared an overlapping set of proteins enriched in telomeric sequence pull-downs (8 overlapping factors out of 12 and 8 hits). Second, within our overlapping set of enriched factors, we detected the previously identified ss telomere-binding proteins POT-1, POT-2, and MRT-131,33,37,38. Lastly, the C. elegans KU heterodimer homologs CKU-70 and CKU-80 were enriched in the screens. In other organisms, such as Saccharomyces cerevisiae, Trypanosoma brucei, Drosophila melanogaster, and Homo sapiens, KU proteins have been shown to associate with telomeres, regulating their length and protecting them from degradation and recombination34,35. The C. elegans homologs were shown to interact with telomeres, but do not seem to have telomere regulatory functions43. However, CKU-70 and CKU-80 were not enriched in the TEBP-1 and TEBP-2 interactome experiments, suggesting that their binding to telomeric DNA occurs independently of the TEBP-1/TEBP-2 complex (Fig. 5 and Supplementary Fig. 6). Alternatively, these factors may be part of the telomeric complex, with no direct interaction with TEBP-1 or TEBP-2.

We identified POT-3 in the background of our LFQ screen (Supplementary Data File 3), supporting the lack of telomeric phenotypes of pot-3 mutants34. Furthermore, a number of factors previously reported to have telomere DNA-binding capability or to regulate telomere length, were not detected or lacked significant enrichment in our quantitative proteomics screen. MRT-2 is a homolog of S. cerevisiae checkpoint gene RAD17 and human RAD1, previously reported to regulate telomere length30. Much like tepb-2 and mrt-1, mrt-2 mutants have shorter telomeres than wild-type and a Mrt phenotype. It is plausible that MRT-2 regulates telomere length beyond the context of direct telomeric binding. PLP-136, HMG-537, and CEH-3738, were previously shown to bind to the C. elegans telomeric sequence in vitro. PLP-1 was enriched in the (AGGTCAn) scrambled control in our qMS screen (Supplementary Data File 3), suggesting that PLP-1 is a general ds DNA binder, and not a specific telomere binder. Furthermore, HMG-5 was detected in the background, and CEH-37 was not detected altogether in our screen (Supplementary Data File 3). Further studies should clarify if and how these factors interact with the telomere complex described in this work.

Discussion
Telomeres and their associated proteins are important to ensure proper cell division. In the popular model nematode C. elegans, only ss telomere-binding proteins were known thus far31,38. Here, we describe a telomeric complex with the paralogs TEBP-1 and TEBP-2 as direct ds telomere-binding proteins. POT-1 seems to bridge the ds telomere-binding module of the complex, comprised of TEBP-1 and TEBP-2, with the ss telomere region. Strikingly, despite the high level of sequence similarity between TEBP-1 and TEBP-2, their mutant phenotypes are divergent.

orthologs. The multiple sequence alignment showed the N-terminal region of tepb genes, the region with similarity to the homeodomains of human and yeast RAP1 (Supplementary Fig. 1d, e and Supplementary Data file 1), is more similar between orthologs than the C-terminal region (Supplementary Data File 2). However, phylogenetic analysis with only the N-terminal region did not produce major differences on tree topology (Supplementary Fig. 7). In order to derive evolutionary relationships between different tepb genes, we evaluated local synteny information. We found a high degree of regional synteny conservation between C. elegans tepb-1 and one of the tepb copies in C. inopinata, C. remanei, C. briggsae, C. nigoni, C. sinica, C. tropicalis, and C. japonica (Table 1 and Supplementary Data file 2). Conversely, tepb-2 did not show any signs of regional synteny across Caenorhabditis species (Supplementary Data file 2), suggesting that the gene duplication event creating tepb-2 occurred after divergence from the C. inopinata species, less than 10.5 million years ago53. Neither of the two tepb orthologs of C. brenneri, C. latens, and C. angaria are in synteny with C. elegans tepb-1 (Supplementary Data file 2).

To determine whether TEBP proteins are generally telomere-binders in the Elegans supergroup, we performed DNA pull-downs, using nuclear extracts prepared from synchronized C. briggsae gravid adults. CBG11106, the only C. briggsae ortholog of tepb-1 and tepb-2, was significantly enriched in the telomere pulldown (Fig. 7b), demonstrating that it can bind to the TTAGGG telomeric repeat. Of note, CBG22248, one of the two C. briggsae orthologs of MRT-1, was also enriched in the telomere pulldown, and CBG16601, the ortholog of POT-1, was just below our significance threshold, suggesting functional similarities to their C. elegans orthologs.

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The fast-evolving paralogs TEBP-1 and TEBP-2 are required for fertility. TEBP-1 and TEBP-2 share 65.4% of their amino acid sequence, which most likely reflects a common origin by gene duplication. Interestingly, the two paralogs TEBP-1 and TEBP-2 interact with each other, and with the same set of factors, i.e., POT-1, POT-2, and MRT-1 (Fig. 5 and Supplementary Fig. 6). This is striking, considering the divergent phenotypes of tebp-1 and tebp-2 mutants: tebp-1 mutants have longer telomeres than wild-type, while tebp-2 animals have shorter telomeres than wild-type and a Mortal Germline. Moreover, while the fertility of tebp-1 and tebp-2 animals is not compromised, tebp-1; tebp-2 double mutants show highly penetrant synthetic sterility irrespective of the temperature the animals are grown at, indicating that TEBP-1 and TEBP-2 contribute to normal fertility (Fig. 4 and Supplementary Fig. 5). The observed synthetic sterility is likely justified by failure to enter and progress through normal mitosis and meiosis, as judged by the under-proliferation of germ cells.

The synthetic sterility of tebp-1; tebp-2 animals is specific to these two paralogs, as other genetic crosses of shorter versus longer telomere mutants did not result in sterile double mutants. The synergistic role of TEBP-1 and TEBP-2 in fertility provide a puzzling contrast with their opposed telomere length mutant phenotypes. We speculate that the requirement of TEBP-1 and TEBP-2 to fertility may be independent of their functions at telomeres. As we have confirmed telomere binding in C. elegans and C. briggsae (species in bold indicate confirmed binding of TEBP proteins to telomeric DNA), it is plausible that their common ancestor was able to bind to telomeres. The gene duplication that generated tebp-2 occurred after the divergence of C. elegans and C. inopinata (marked as orange stripe), followed by division, or diversification, of functions of these two paralogs (TEBP-1: yellow hexagon, TEBP-2: red hexagon).

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speculate that tebp-1 and tebp-2 originated from an ancestor Caenorhabditis tepg gene required for fertility with the ability to bind ds telomeric repeats (Fig. 7c). The tebp-1 ancestor was duplicated after the divergence of C. inopinata and C. elegans, 10.5 million years ago53, likely initiating a process of functional diversification of tepb-1 and tepb-2.

Given their possible recent divergence, in evolutionary terms the 65.4 % protein sequence similarity observed between the protein sequences of TEBP-1 and TEBP-2 is actually fairly low. This likely reflects fast evolution of TEBP-1 and TEBP-2, in line with the known fast evolution as suggested for other telomere-binding proteins60. While it is tempting to establish evolutionary relationships with vertebrate TRF1 and TRF2 proteins, TEBP-1/TEBP-2 and TRF1/TRF2 are not homologs. In addition, TRF1 and TRF2 are binding to telomeric DNA via C-terminal myb-domains61, while DNA binding in TEBP-1 and TEBP-2 occurs N-terminally. However, on the functional level, similarity between C. elegans TEBP-1/TEBP-2 and vertebrate TRF1/TRF2, potentially reflecting convergent evolution between two phylogenetically independent sets of telomere-binding paralogs is possible, but needs further investigation.

A telomere complex in actively dividing tissues in homeostasis.

| Table 1 Synteny analysis of tebp orthologs in other Caenorhabditis species. |
|-----------------|----------------|----------------|
| tebp ortholog  | Synteny with tebp-1 | Synteny with tebp-2 |
| 10007010 (C. inopinata) | - | - |
| 20164200 (C. inopinata) | + | - |
| FLB2_06185 (C. remanei) | + | - |
| FLB3_05550 (C. latens) | - | - |
| CBG1106 (C. briggsae) | + | - |
| Cni-PFS-2.3 (C. nigi) | + | - |
| g13401 (C. sinica) | - | - |
| CBN00774 (C. brenneri) | - | - |
| CBN07368 (C. brenneri) | - | - |
| gi5680 (C. tropicalis) | - | - |
| gi5070 (C. tropicalis) | - | - |
| CJA1830 (C. japonica) | + | - |
| FLB3_22916 (C. latens) | - | - |
| FLB3_22905 (C. latens) | - | - |
| FLB2_20056 (C. remanei) | - | - |
| gi5539.13 (C. angaria) | - | - |
| gi1959 (C. angaria) | - | - |

Overall, these data indicate that the telomere-binding proteins are present in C. elegans and C. briggsae, and suggest that they may have similar functions in these species. The synteny analysis also supports the idea that the telomere-binding proteins have evolved conserved domains that are important for their function.

Methods

C. elegans nuclear-enriched protein extract preparation. Nuclear extract preparation of gravid adult worms was done as described68. The worms were synchronized by bleaching and harvesting at the gravid adult stage by washing them off the plate with M9 buffer. After washing the worms in M9 buffer for 4 times, they were pulled by centrifugation at 600 x g for 4 min, M9 buffer was removed and extraction buffer (40 mM NaCl, 20 mM MOPS pH 7.5, 90 mM KCl, 2 mM EDTA, 0.5 mM EGTA, 10% Glycerol, 2 mM DTT, and 1x complete protease inhibitors Roche) was added. Worms resuspended in extraction buffer were frozen in liquid nitrogen. The resulting pellets were ground to a fine powder in a pre-cooled mortar and transferred to a pre-cooled glass douncer. When thawed, the samples were sheared with 30 strokes, piston B. The worm suspension was pipetted to pre-cooled 1.5 ml reaction tubes (1 ml per tube) and cell debris, as well as unsheared worms were pulsed by centrifugation at 200 x g for 5 min at 4 °C for two times. To separate the cytoplasmatic and nuclear fractions, the supernatant was spun at 2000 x g for 5 min at 4 °C. The resulting pellet containing the nuclear fraction was washed twice by resuspension in extraction buffer and subsequent centrifugation at 2000 x g for 5 min at 4 °C. After the washing steps, the nuclear pellet was resuspended in 200 µl buffer C + (420 mM NaCl, 20 mM Hepes/KOH pH 7.9, 2 mM MgCl2, 0.2 mM EDTA, 20% Glycerol, 0.1% Igepal CA 630, 0.5 mM DTT, 1x complete protease inhibitors). Nuclear extract of gravid adult worms of C. briggsae was prepared as described above.

Oligonucleotides. All oligonucleotides used throughout this manuscript (cloning, sequencing, DNA pulldowns, fluorescence polarization etc.) are listed in Supple-mentary Data file 4 with their name and sequence.

DNA pulldowns

Preparation of biotinylated DNA for pulldown experiments. Biotinylated telomeric and control DNA for the DNA pulldown for detection of telomeric interactors was prepared as previously published66,67. In short, 25 µl of 10-mer repeat oligo-nucleotides of either telomeric or control sequence were mixed 1:1 with 25 µl of their respective reverse complement oligonucleotide and 10 µl annealing buffer (200 mM Tris-HCl, pH 8.0, 100 mM MgCl2, 1 M KCl). The mixture was brought to...
100 µl final volume with H₂O, heated at 80 °C for 5 min, and left to cool. Once at room temperature (RT), the samples were supplemented with 55 µl H₂O, 20 µl 10x T4 DNA ligase buffer (Thermo Scientific), 10 µl PEG 6000, 10 µl 100 mM ATP, 2 µl 1 M DTT and 5 µl T4 Polynucleotide Kinase (NEB, 10 U/µl, #M0201) and left at 37 °C for 2 h to concatenate. Finally, 4 µl of T4 DNA Ligase (Thermo Scientific, 5 U/µl, #E0011) were added and the samples incubated at RT overnight for ligation and polymerization. The ligation process was monitored by running 1 µl of the reaction on a 1% agarose gel. The samples were cleaned by phenol-chloroform extraction. For this, 1.0 vol. of H₂O and 200 µl of Phenol/Chloroform/Isoamyl Alcohol (25:24:1; pH 8; Invitrogen, #15593049) was added to the mixture, vortexed and centrifuged at 16,000 x g for 2 min. After centrifugation the aqueous phase was transferred to a new tube and the DNA was precipitated by addition of 1 M Ethanol and incubation at −20 °C for 30 min. Afterwards the suspension was centrifuged at 16,000 x g for 45 min at 4 °C. The resulting DNA pellet was resuspended in 74 µl H₂O and 10 x 100 µl Klenow-fragment reaction buffer (Thermo Scientific), 10 µl 0.4 mM Biotin-7-ATP (Jena Bioscience, #NU-835-BIO) and 6 µl Klenow-fragment exo- polymerase (Thermo Scientific, 5 U/µl, #EP0422) added.

Biocytin was carried out by incubation at 37 °C overnight. The reaction was cleaned up by size-exclusion chromatography using MicroSpin Sephadex G-50 columns (GE Healthcare, #G27-3300-01).

Pulldown experiments. Biotinylated DNA and Dynabeads™ MyOne™ Streptavidin C1 (Thermo Scientific, #65001) were mixed with PBB buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.5% NP-40, 5 mM MgCl₂, 1 mM DTT) and incubated at room temperature for 15 min on a rotating wheel to immobilize the DNA on the beads. After three washes with PBB buffer, the DNA coupled beads were resuspended in PBB buffer and Salmon sperm (10 mg/ml, Ambion, #AM9680) was added:1100 as competitor for unspecific DNA binding. The pulldowns were performed with different amounts of protein extract (see below) and incubated at 4 °C on a rotating wheel for 90 min. Following incubation the beads were washed three times with PBB buffer and resuspended in 1x Loading buffer (4x NuPAGE LDS sample buffer, Thermo Scientific, #NP0008) supplemented with 100 mM DTT. For elution, the samples were boiled at 70 °C for 10 min and afterwards loaded on an in-house packed C18 column (500 µl, 25 cm long) with 75 µm inner diameter) for reverse-phase chromatography. The Easy-nLC 1000 system (Thermo Scientific) was mounted to a Q Exactive Plus mass spectrometer (Thermo Scientific) and peptides were eluted from the column in an optimized 2 h (pulldown) gradient from 2 to 40% of 80% MS grade acetonitrile/0.1% formic acid solution at a flow rate of 225 nL/min.

The mass spectrometer was used in a data-dependent acquisition mode with one MS full scan and up to ten MS/MS scans using HCD fragmentation. All raw files were processed with MaxQuant (version 1.5.2.8) and searched against the C. elegans Wormbase protein database (Version WS269, as well as the Ensembl Bacteria E. coli REL606 database (version from September 2018) for proteins from the feeding experiments. Carbamidomethylation (Cys) was set as fixed modification, while oxidation (Met) and protein N-acetylation were considered as variable modifications. For enzyme specificity, trypsin was selected with a maximum of two miss-cleavages. LFQ quantification (without fast LFQ) using at least 2 LFQ ratio counts and a 50% confidence when the LFQ ratio was above 1.0 was used for peak detection. Fractions and conditions were indicated according to each experiment. Data analysis was performed in R using existing libraries (ggplot2-v 3.2.1, ggrepel-v 0.8.1, stats-v 3.5.2) and in-house scripts. Protein groups reported by MaxQuant were filtered removing known contaminants, protein groups only identified by site and those identified as reverse were removed. Data were plotted as volcano plots. The lower end of LFQ values using random values from a beta distribution fitted at 0.2–2.5%. For statistical analysis, p-values were calculated using Welch’s t-test. Enrichment values in the volcano plots represent the mean difference of log2 transformed and imputed LFQ intensities between the telomere and the control enriched proteins. Peptide labels created by the dimethyl-labeling reaction were extracted in the MaxQuant software. "N-terminal Dimethyl 0.0" and "Dimethyl 0.5" for modification, while oxidation (Met) and protein N-acetylation were considered as variable modifications. For enzyme specificity, trypsin was selected with a maximum of two miss-cleavages. LFQ quantification (without fast LFQ) using at least 2 LFQ ratio counts and a 50% confidence when the LFQ ratio was above 1.0 was used for peak detection. Fractions and conditions were indicated according to each experiment. Data analysis was performed in R using existing libraries (ggplot2-v 3.2.1, ggrepel-v 0.8.1, stats-v 3.5.2) and in-house scripts. Protein groups reported by MaxQuant were filtered removing known contaminants, protein groups only identified by site and those identified as reverse were removed. Data were plotted as volcano plots. The lower end of LFQ values using random values from a beta distribution fitted at 0.2–2.5%. For statistical analysis, p-values were calculated using Welch’s t-test. Enrichment values in the volcano plots represent the mean difference of log2 transformed and imputed LFQ intensities between the telomere and the control enriched proteins. Peptide labels created by the dimethyl-labeling reaction were extracted in the MaxQuant software. "N-terminal Dimethyl 0.0" and "Dimethyl 0.5" for modification, while oxidation (Met) and protein N-acetylation were considered as variable modifications.
TEBP-1-His, and TEBP-2-His, were expressed in Rosetta 2 (DE3) pLysS competent cells (Novagen, #71401). An overnight culture was grown in LB containing the respective antibiotic. A gelled culture was inoculated and after reaching mid-log growth at 37 °C, the cultures were induced with 1 mM IPTG. Cells were grown at 18 °C and harvested after 24 h. IPTG-induced or auto-induction cultures were pelleted in 30 ml reaction tubes by centrifugation at 4500 x g after growth and lysed according to the protocol for the respective downstream use.

POST-2 expression pellets were resuspended in Tris buffer (50 mM Tris/HCl pH 7.5, 100 mM NaCl, 10 mM MgCl₂, 1x EDTA-free protease inhibitor (Roche, #493132001)) and divided into 2 ml flat lid micro tubes containing 0.1 mm zirconia beads (Carl Roth, #N0331). Lysis of the cells was achieved with a FastPrep® -24™ Classic (MP Biomedicals, #1160045000) using the setting m/s to 30 s for a few times. In between the disruption cycles the samples were centrifuged at 21,000 x g for 2 min to pellet debris, followed by an incubation on ice for 5 min before the second cycle. After lysis the suspension was centrifuged at 21,000 x g for 10 min at 4 °C.

TEBP-1 and TEBP-2 expression pellets were lysed via sonication with a Branson Sonifier 450 (duty cycle: 50%, output control: 3, 3.5 min with 5 mm tip) in lysis buffer (25 mM Tris-HCl pH 7.5, 300 mM NaCl, 20 mM imidazole) with 1 mM DTT, and protease inhibitor cocktail tablets (Roche, #493132001). Lysates were centrifuged at 46,130 x g for 45 min at 4 °C. For both preparation methods the supernatant was afterwards transferred to fresh reaction tubes. His-MBP tagged TEBP-1 and TEBP-2 fragments were extracted in E.coli Arctic Express DE3 cells (Agilent, #230192). Cells were grown overnight in 5 ml LB supplemented with the respective antibiotic for the expression vector. Next day the expression culture was inoculated from the overnight culture and grown to mid-log phase at 30 °C, and then induced with 1 mM IPTG. Cultures were incubated at 12 °C and harvested after 24 h. The pellet was resuspended in binding buffer (20 mM Tris-HCl pH 7.5, 500 mM NaCl, 50 mM imidazole) with 1 mM DTT, complete protease inhibitor cocktail tablets (Roche, #493132001), and 100 µg DNase I (NEB, #M0303S). Cells were lysed using a Branson Sonifier (duty cycle: 50%, output control: 4, 6 min (3 min sonication, 3 min ice, 3 min sonication) with 9 mm tip). Lysates were cleared at 46,130 x g for 10 min at 4 °C, and used for subsequent assays.

Protein expression, purification, and fluorescence polarization assay. E.coli Arctic Express DE3 cells (Agilent, #230192) were grown overnight in 5 ml LB supplemented with the respective antibiotic for the expression vector. Next day the expression culture was inoculated from the overnight culture and grown to mid-log phase at 30 °C, and then induced with 1 mM IPTG. Cultures were incubated at 12 °C and harvested after 24 h. The pellet was resuspended in binding buffer (20 mM Tris-HCl pH 7.5, 500 mM NaCl, 50 mM imidazole) with 1 mM DTT, complete protease inhibitor cocktail tablets (Roche, #493132001), and 100 µg DNase I (NEB, #M0303S). Cells were lysed using a Branson Sonifier (duty cycle: 50%, output control: 4, 6 min (3 min sonication, 3 min ice, 3 min sonication) with 9 mm tip). Lysates were cleared at 46,130 x g for 10 min at 4 °C, and used for subsequent assays.

Immunoprecipitation (IP). IP:s with FLAG-tagged protein were performed with Protein G magnetic beads (Invitrogen) Dengabe Protein G; #10004D) and α-FLAG antibody (Monoclonal ANTI-FLAG™ M2 antibody produced in mouse, Sigma Aldrich, #F3165). Per IP; 30 µl of beads were used and washed three times with 1 ml Wash Buffer (25 mM Tris/HCl pH 7.5, 150 mM NaCl, 1.5 mM MgCl₂, 1 mM DTT, 1 complete Mini protease inhibitor tablet per 50 ml). The beads were resuspended in 450 µl Wash Buffer and up to 1 mg of complete protein extract from the respective C. elegans strains was added. Finally, 2 µg of FLAG antibody were added and the samples were incubated for 3 h, rotating at 4 °C. After the incubation, the samples were washed three to five times with 1 ml Wash Buffer (see washing steps before), the beads were resuspended in 1x LDS/DTT, and the samples were boiled at 95 °C for 10 min. For mass spectrometry, IP:s were prepared in quadruplicates per strain/condition. When doing the IP with Sm nuclease, the wash buffer was supplemented with 0.05% of recombinant endonuclease from Serratia marcescens, or Sm nuclease, produced by the IMB’s Protein-Production Core Facility.

FLAG IP. IP:s with FLAG-tagged protein were performed with Protein G magnetic beads (Invitrogen) Dengabe Protein G; #10004D) and α-FLAG antibody (Monoclonal ANTI-FLAG™ M2 antibody produced in mouse, Sigma Aldrich, #F3165). Per IP; 30 µl of beads were used and washed three times with 1 ml Wash Buffer (25 mM Tris/HCl pH 7.5, 150 mM NaCl, 1.5 mM MgCl₂, 1 mM DTT, 1 complete Mini protease inhibitor tablet per 50 ml). The beads were resuspended in 450 µl Wash Buffer and up to 1 mg of complete protein extract from the respective C. elegans strains was added. Finally, 2 µg of FLAG antibody were added and the samples were incubated for 3 h, rotating at 4 °C. After the incubation, the samples were washed three to five times with 1 ml Wash Buffer (see washing steps before), the beads were resuspended in 1x LDS/DTT, and the samples were boiled at 95 °C for 10 min. For mass spectrometry, IP:s were prepared in quadruplicates per strain/condition. When doing the IP with Sm nuclease, the wash buffer was supplemented with 0.05% Sm nuclease (as indicated above).

Western blot. Protein samples were boiled at 70 °C for 10 min and loaded on a 4–12% Bis-Tris gel (NuPAGE, Thermo Scientific, #NP0321), running at 150–180 V for 1–2 h in 1x MOPS, then transblotted to a nitrocellulose membrane (Amersham Protran, VWR, #10600002) equilibrated in transfer buffer as well. Membrane and gel were stacked with pre-wet Whatman paper (GE Healthcare-Whatman, #WHA10428982) and immersed in a blotting tank (Bio-Rad) filled with ice-cold transfer buffer and additionally cooled with a cooling element. The proteins were blotted at 300 mA for 60–120 min depending on the size. If blotted for 90–120 min for larger proteins, the transfer was carried out with a blotting tank on ice to keep the temperature. After blotting, the membranes were further prepared according to the respective antibody protocol.

Anti-His antibody. Membranes were blocked in Blocking Solution (PentaHis Kit, Qiagen, #34460) for 1 h at room temperature. After 3 minutes washes in TBS-T (1x TBS, 0.1% Tween-20, 0.5% Triton X-100) the membranes were incubated with the Anti-His-HRP conjugated antibody in a dilution of 1:1000 in Blocking Solution for 1 h at room temperature. The membranes were washed with TBS-T and incubated with ECL Western Blot reagent (Thermo Scientific™ SuperSignal™ West Pico Plus Chemiluminescent Substrate, #15626144; mixed 1:1) for detection. Western blot ECL detection was performed with the ChemiDoc XRS+ system (BioRad, Software: Image Lab 5.2.1).

Anti-GFP, Anti-FLAG, and Anti-Actin antibodies. Western blot analysis was performed using the following primary antibodies: an anti-GFP antibody (Roche, Anti-GFP from mouse IgGx (clones 7.1 and 13.1), #1181446001; 1:1000 in Skim Milk solution), an anti-FLAG antibody (Sigma-Aldrich, mouse Monoclonal ANTI-FLAG™ M2 antibody, # F3165; 1:5000 in Skim Milk solution), and an anti-Actin antibody (Abcam, mouse anti-Actin, #A2066; 1:5000 in Skim Milk solution). After blotting, membranes were blocked in Skim Milk solution (5x PBS, 0.1% Tween-20, 5% (w/v) Skim Milk Powder) for 1 h at room temperature. The
incubation with the primary antibody was carried out at 4 °C, rotating overnight. Membranes were washed in PBS-T (1x PBS, 0.1% Tween-20) three times for 10 min. Blots were blocked with an HRP-linked (for-FLAG and anti-GFP with Cell Signaling Technology, anti-mouse IgG, #7076; 1:1,000 dilution in skim milk solution) for 1 h rotating at room temperature. Following three washes in PBS-T the membranes were incubated with ECL solution (Thermo Scientific ™ SuperSignal ™ West Pico PLUS Chemiluminescent Substrate, #15626144; mixed 1:1 for detection). Western blot ECL detection was performed with the ChemiDoc XRS + system (BioRad, Software: Image Lab 5.2.1). Incubation with Anti-Actin antibody was typically performed after detection of GFP/FLAG and subsequent washes.

Antibody protocol for co-IPs (LI-COR antibodies)

For co-IP experiments, we first probed the IP bait with HRP-linked secondary antibodies, as described above. Then, we probed for the co-IP using LI-COR secondary antibodies. After incubation with primary antibody, as described above, membranes were washed and incubated with secondary antibodies compatible with the LI-COR SYSTEM (FLAco/ GEP; Licor IRDye® 680RD Donkey anti-Mouse IgG (H+L), #926–68702; Actin: Licor IRDye® 800CW Donkey anti-Rabbit IgG (H+L), #926–32212; 1:15,000 in skim milk solution) for 1 h at room temperature. After three additional washes with PBS-T, the membranes were imaged using an Odyssey CLx scanner and processed using Image Studio software (LI-COR, Version 3.1).

E. coli culture and strains.

E. coli was cultured under standard conditions on Nematode Growth Medium (NGM) plates seeded with E. coli OP50 bacteria. For proteomics experiments, animals were grown on OP50 high-density plates (adapted from ref. 72). In specific, the yolk of commercially available chicken eggs were isolated, added to LB medium (50 ml per egg yolk) and thoroughly mixed. Subsequently, the mix was incubated at 65 °C for 3 h. Prior to bacterial culture the mix was added to the mix (10 ml per egg), after the yolk-LB mixture cooled down. This preparation was poured into 9 cm plates (10 ml per plate) and plated and decanted the next day. Plates remained for 2–3 days at room temperature, for further bacterial growth and drying.

Animals were grown at 20 °C, except when noted. The standard wild-type strain used in this study was N2 Bristol. Strains used and created in this study are listed in Supplementary Table 1.

Fertility assays.

For brood size counts the homozygous single mutants, L3 worms were isolated, per strain and were grown either at 20 or 25 °C. After reaching adulthood, worms were transferred to a new plate every day, until no eggs were laid in 2 consecutive days. Viable progeny was counted approximately 24 h after removing the parent. For the experiment shown in Fig. 4d, e, a, c, between tebp-1(xf133) males and tebp-2(xf131) hermaphrodites was performed, the genotypes of the F1 and F2 were confirmed by PCR genotyping. L2/L3 progeny of F2 tebp-1 (xf133) +; tebp-2 (xf131) mothers were isolated and grown at 20 °C, or 25 °C. During adulthood, the viable brood size was counted as mentioned above. After the assays, both 2 days after egg laying stopped. For all brood size experiments, worms that died before egg laying terminated, e.g., by dehydration on the side of plate, were excluded from the analysis.

Mortal germline assay.

All strains used in the Mortal Germline assay were out-crossed with wild-type N2 two times before the experiment. Six L3 larvae of the chosen strain were picked per plate (n = 15 plates per strain) and grown at 25 °C. Six L3 larvae were transferred to a fresh plate every 5 days (equivalent to two generations). This procedure was followed until plates were scored as sterile, when the six worms transferred failed to produce six offspring to further isolate, on 2 consecutive transfer days.

pgl-1::mTagRfp-T; tebp-1 x pgl-1::mTagRfp-T; tebp-2 x pgf-2 and definition of categories of germline defects.

We crossed pgl-1::mTagRfp-T; tebp-1 males with pgl-1::mTagRfp-T; tebp-2 hermaphrodites. F1 crosses were confirmed by PCR genotyping. 300 F2 progeny were single and left to self-propagate. After genotyping F2 worms, we isolated 60 F3 worms from three different tebp-1 (xf133) tebp-2 (xf131) +; tebp-2 (xf131) mothers, as well as 10 F3 worms from two different single mutant mothers as controls. Additionally, all synthetic sterility escape progeny from tebp-1; tebp-2 double-homologous worms were single to check their fertility. Germline health, as well as growth and other phenotypes for all singled worms were determined at 20 °C. Co-converted F1 progeny were screened for insertions by PCR. Successful co-conversion was transplanted to the editing site to generate d10-entry strains, which in turn served as reference strains for further injections. DNA mix was injected in both gonad arms of 10–25 1-day-old adult hermaphrodites maintained at 20 °C. Co-converted F1 progeny were screened for insertions by PCR. Successful co-conversion events were confirmed by Sanger sequencing. All generated mutant worms were outcrossed at least two times at prior any further cross or analysis. CRISPR-Cas9 genome editing reagents and DNA injection mixes are listed in Supplementary Data file 5. The pgl-1::mTagRfp-T is described elsewhere.20

Creation of transgenic worms using MosSCI.

A TEBP-2::GFP fusion transgene was produced as previously described16, and as indicated in www.wormbuilder.org. Animals were derived from the strain EG6699, which was obtained, in order to produce a C. elegans strain carrying ttt5605 on LGII. The injection mix contained all the recommended concentrations, including 50 ng/µl of a repair template containing the tebp-2::gfp sequence. Selection was performed as recommended in www.wormbuilder.org.45

Extraction of genomic DNA from C. elegans.

Mixed-staged animals were washed off plates with M9 and washed two to three more times in M9. Next, worms were resuspended in Worm Lysis buffer (WLB: 0.2 M NaCl, 0.1 M Tris/HCl pH 8.5, 50 mM EDTA, 0.3% SDS) and aliquoted in 250 µl samples. For genomic DNA extraction the aliquots were brought to a final volume of 500 µl with WLB and Proteinase K (30 µg/ml). To lyse the worms, the samples were incubated at 65 °C at 150 rpm for 1 h and then cooled on ice for >2 h until all cellular components were dissolved. Subsequently, the mix was incubated at 65 °C for 2 h and 30 s. The sample was centrifuged at 12,000 × g for 5 min to pellet debris and the supernatant was transferred to a fresh tube. Afterwards, 500 µl of Phenol:Chloroform:
Isoamyl alcohol were added, the samples shaken vigorously for 30 s and spun down at 16,000 g for 5 min. Additionally, 500 µl of chloroform were added to the samples and centrifuged for 30 sec at 16,000 x g. The aqueous phase of the samples was transferred to fresh 2 ml reaction tubes and 50 µg RNase A were added to digest the RNA. The tubes were inverted once and incubated at 37 °C for >1 h. After RNA digestion the samples were again purified by phenol:chloroform:isoamyl alcohol and chloroform addition (as before). The aqueous phase was transferred to fresh tubes and the DNA was precipitated with 350 µl isopropanol for >15 min at ~80 °C. To pellet the DNA, the samples were centrifuged at 21,000 × g for 20 min at 4 °C. The supernatant was carefully removed and the DNA pellet washed once with 1 ml of ice-cold 70% ethanol and spun at 21,000 × g for 5 min at 4 °C. Washing was repeated once the sample still contained some of phenol. After washing the supernatant was completely removed, the pellet air dried for ca. 10 min, and resuspended in 20 µl H2O. To fully resuspend the DNA, the samples were kept at 4 °C overnight and mixed again the next day.

Telomere Southern blot

For denatured telomere Southern blot 15 µg of C. elegans genomic DNA were digested in 80 µl total volume with 40 U HinfI (New England Biolabs, #R0155) and RsaI (New England Biolabs, #R0167), respectively. The digestion was incubated at 37 °C overnight and the next day additional 10 U of each enzyme were added and the samples incubated 1–2 h further. Afterwards the samples were evaporated in a Concentrator Plus at 45 °C to end up with a volume of 20–30 µl and supplemented with 2x DNA loading dye. A 0.6% agarose gel was prepared (with 1x TBE and 16 µl SYBR Safe DNA stain, Thermo Fisher Scientific, #S33102) and the samples loaded in 2% TBE/EDTA, 0.3 M NaCl, 0.03 M EDTA, 3 x 10^{-7} M Tris (pH 8.0) (Supplementary Fig. 5a) two embryo size-fractionated samples were co-transformed in PJ69-4 by TTAGGRC reverse complement triple repeat (GCCCTAA). The probe was radioactively labeled with 3 µl 32P-[y]-ATP by a polynucleotide Kinase reaction and cleaned up using a Microspin Sephadex G-50 column (GE Healthcare, #GE27-5330-01). The labeled oligonucleotide was denatured at 95 °C for 10 min and mixed with 20 µl fresh hybridization buffer. This mix was added to the membrane after removing the previous buffer and incubated for 3.5 days rotating at 42 °C.

After hybridization the membrane was washed by first rinsing it twice with Wash Buffer 1 (2x SSC, 0.1% SDS), then incubating it twice for 5 min in 20 µl Wash Buffer 2. For the last wash, the membrane was incubated for 2 min in Wash Buffer 2 (0.2x SSC, 0.1% SDS), then rinsed in 2x SSC to re-equilibrate the salt concentration. The membrane was dried with a white paper for 3 min and incubated in a hybridization oven. The oligonucleotide used for detection was optimized after the work of Seo and Lee89. Per strain, 100 gravid adults were processed using Fiji. The images were optimized using Fiji.

Microscopy

Co-localization microscopy. Strains carrying TEBP-1::GFP or TEBP-2::GFP were crossed with strain YA1197 expressing P::HCX PL APO 63x water objective (NA 1.2), Leica hybrid detectors (HyD), and the acquisition software Leica LAS AF. The images stacks were composed by a sequence of z-stack was converted to a binary mask and using the 3D OC Options menu volume, mean gray values and integrated density of the FISH image were calculated. Additionally, the 3D Object counter menu was used and the filters set to a minimum of 2. The values obtained by this analysis were averaged over several images of either germlines or embryos of the same strain and used for quantitative comparison of telomere length. For comparison, all values obtained for the mutant strains were scaled relative to the average of the wild type values. The barplots were created using R with standard and publicly available scripts (RCoolr, Rg3 activation and DNA-binding domain plasmid pairs were co-transformed in P69–4e. The resulting transformants were resuspended in ddH2O and pinned on SC Trp-Leu-, SC Trp-Leu-His–, and SC Trp-Leu-His–Ade– plates. For Fig. 6a a random additional of plasmid transformation was performed, as a biological duplicate, and the results were identical. Colonies were picked with a ChemiDoc XRS+ (Bio-Rad) using lysis buffer without Triton X-100 as running buffer..

Size-exclusion chromatography. Size-exclusion chromatography was performed as previously described.112 The respective G4 activation and DNA-binding domain plasmid pairs were co-transformed in P69–4e. The resulting transformants were resuspended in ddH2O and pinned on SC Trp-Leu–, SC Trp-Leu–His–, and SC Trp-Leu–His–Ade– plates. For Fig. 6a a random additional of plasmid transformation was performed, as a biological duplicate, and the results were identical. Colonies were picked with a ChemiDoc XRS+. The resulting post- and Supplementary Fig. 6i. Yeast two-hybrid assay. Yeast two-hybrid assays were conducted in the yeast strain P69–4e as described before.62 The respective G4 activation and DNA-binding domain plasmid pairs were co-transformed in P69–4e. The resulting transformants were resuspended in ddH2O and pinned on SC Trp-Leu–, SC Trp-Leu–His–, and SC Trp-Leu–His–Ade– plates. For Fig. 6a a random additional of plasmid transformation was performed, as a biological duplicate, and the results were identical. Colonies were picked with a ChemiDoc XRS+. The resulting post- and Supplementary Fig. 6i.

Size-exclusion chromatography. Size-exclusion chromatography was performed as previously described.112 The first run (Supplementary Fig. 5a) two embryo samples were prepared and combined. Using a centrifugal filter with a 10 KDa cut-off (Merck, Amicon Ultra 0.5 ml, 10 K, #UFC9010) the sample was concentrated to a final volume of 550 µl. Between 3.6 and 3.8 mg of total extract was separated on a Superose 6 10/300 GL column (GE Healthcare, 17517201) operated on a NGC Quest System (Bio-Rad) using lysis buffer without Trp-Leu-T for running buffer (25 mM Tris/HC1 pH 7.5, 150 mM NaCl, 1.5 mM MgCl2, 1 M DTT, protease inhibitors). Five hundred microliter fractions were collected according to the sample with the lowest FISH intensity. For analysis, images were loaded in Image J/Fiji and the channels split into the DAPI and red channel. A mask of the image was created to infer the volume of the imaged object.

The threshold function of the software was used with activated plugins for identification of round objects (Otsu). After setting the threshold for the image in the histogram settings, the z-stack was converted to a binary mask and using the 3D OC Options menu volume, mean gray values and integrated density of the FISH foci were calculated. Additionally, the 3D Object counter menu was used and the filters set to a minimum of 2. The values obtained by this analysis were averaged over several images of either germlines or embryos of the same strain and used for quantitative comparison of telomere length. For comparison, all values obtained for the mutant strains were scaled relative to the average of the wild type values. The barplots were created using R with standard and publicly available scripts (RCoolr).
The protein sequences of *C. elegans* TEBP-1 and TEBP-2 were extracted from Wormbase (WS275). These sequences were used separately as queries for Wormbase BLASTP search in the available genomes. orthologs of TEBP-1 and TEBP-2 were defined based on two criteria: (1) BLASTP hit had an E-value lower than 1.00e-15; and (2) reciprocal BLASTP of the hit, querying the *C. elegans* proteome, resulted in TEBP-1 and TEBP-2 as top hits. Sequences of the identified orthologs were obtained from Wormbase (WS275) and Wormbase ParaSite (WBS514/WS271). The list of identified orthologs and BLASTP results can be found in Supplementary Data file 2 (sheet 1).

**Phylogenetic and synteny analysis.** The protein sequences of *C. elegans* TEBP-1 and TEBP-2 were used for multiple sequence alignment using MAFFT, version 7.429. Alignment was performed using default settings, including an automatic determination of best alignment strategy, which provided the L-INS-I result90. Multiple sequence alignment can be found in Supplementary Data file 2 (sheet 2). Then, the protein sequence alignment in fasta format was used as an input for IQ-TREE version 1.6.125, with branch supports obtained with ultrafast bootstrap90. IQ-TREE was first run to determine the best fit substitution model, which was VT+F+R3. Then, alignment was repeated with the following parameters: -redo -m VT+F+R3 -b 1000 -o Cang_2012_03_13_01061.g15393.t3_Can, where -m is the best fit model, -b is the number of bootstrap replicates, and -o represents the defined output. The tree file was visualized in FigTree version 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/). The *C. angaria* TEBP orthologs were used as outgroups, as this species is not part of the *Elegans* and *Ljnacca* groups, according to recent phylogenetic studies53. To create an additional tree with the Nematoda regional only the initial multiple sequence alignment was trimmed to the 600 initial alignment positions. The alignment of this region (with similarity to the homoeodomain of RAPI1) was substantially more reliable, as assessed by higher GUIDANCE2 scores98. Using this edited alignment, another tree was constructed as described above. IQ-TREE best fit model parameters used: -m VT+F+G4 -b 1000 -o Cang_2012_03_13_01061.g15393.t3_Can

We defined local synteny across species as the maintenance of linkage in at least one of the neighboring genes upstream and downstream of the respective *tebp* gene. We used two different strategies to determine synteny. (1) Synteny was determined by navigating genome browser tracks through regions containing *tebp* orthologs, using Wormbase ParaSite (WBS514/WS271). Currently annotated genes, adjacent to *tebp* orthologs, were selected, their predicted protein sequences were retrieved and BLASTP was performed in the *C. elegans* genome to find the corresponding ortholog. Results are summarized in Supplementary Data file 2 (sheet 3). The protein sequences obtained were subsequently reciprocal BLASTP of TEBP-1 and TEBP-2 were used as an entry for Wormbase ParaSite BioMart tool (https://parasite.wormbase.org/biomart). We recouped the neighboring 13 genes upstream and 13 genes downstream, and, with the resulting gene ID list, we determined a set of orthologous genes with the following series of ‘Output attributes’: gene stable ID, chromosome/scaffold, start (bp) and end (bp) coordinates that were to be listed in the result from ten available complete *Caenorhabditis* genomes. Subsequently, we filtered only those genes that share the same chromosome/scaffold with the *tebp* orthologous gene, finally, we evaluate if the enlarged group meets our definition of local synteny. We repeated this process taking each of the *tebp* genes in the ten species as a reference and evaluated the filtered groups for local synteny. In the specific case of *C. remanei*, Wormbase ParaSite provides three different assemblies: PRJNA248909, PRJNA248911 and PRJNA353967. The latter was the only assembly where we were able to identify synteny of *tebp-1* with BioMart, although we could verify it manually for PRJNA248911. Results are summarized in Supplementary Data file 2 (sheet 4). This strategy was not applicable to *C. angaria*, as the genome of this species is not implemented in Wormbase ParaSite BioMart.

**RNA extraction and library preparation.** RNA was extracted as described47. Synchronized young adult animals were frozen in 50–100 µl of H2O after harvest. After thawing, 500 µl TRIzol LS reagent (Invitrogen, # 10296010) was added and the worms were lysed with six freeze-thaw cycles (frozen in liquid nitrogen for ca. 30 s), then thawed for 2 min in sodium dodecyl sulfate. Supernatant was transferred to a fresh tube, mixed 1:1 with 100% ethanol and the mix was transferred to a column of the Direct-zol RNA MiniPrep Plus Kit (Zymo Research, #R2070). The following purification steps were done according to manufacturer’s instructions, including the recommended in-column DNase I treatment for 25–40 min. RNA samples were eluted in 30–32 µl of RNAfree H2O.

Library preparation for mRNA sequencing was performed with Illumina’s TruSeq stranded mRNA LT Sample Prep Kit following Illumina’s standard protocol (Part # 15031047 Rev. E). Libraries were prepared by using only ¼ of the reagents with a starting amount of 250 ng and they were amplified in ten PCR cycles. Libraries were profiled in a High Sensitivity DNA on a 2100 Bioanalyzer (Agilent technologies) and quantified using the Qubit dsDNA HS Assay Kit, in a Qubit 2.0 Flurometer (Life technologies). Libraries were pooled in an equimolar ratio and sequenced on one NextSeq 500 HighOutput flowcell, SR for 1 x 75 cycles plus 1 x 7 cycles for index read.

**mRNA read processing and mapping.** The library quality was assessed with FastQC (version 0.11.8) before alignment against the *C. elegans* genome assembly WBCele235 and a custom.GTF file, which included gene annotations from *C. elegans* (Wormbase, c_elegans.PRJNA13758.WS269) and *E. coli* (EnsemblBacteria, Escherichia_coli_b_str rel006.ASM1798v1). Alignment was performed with STAR aligner101 version 2.6.1b. Reads mapping to annotated features in the custom.GTF file were counted with featureCounts102 version 1.6.2 using featureCounts functionality. Counts aligning to *E. coli* were removed at this point from downstream analysis. Coverage tracks were generated with deepTools103 version 2.2.1.7 and plotted using Gviz104 on an R framework (R Core Team 2018).

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability.** The datasets supporting the conclusions of this article are available in the ProteomeXchange Consortium via Pride repository, PXD019241; and in the SRA, BioProject PRJNA630690.

**Code availability.** Code is available upon request.

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#UCFS5010. The samples were supplemented with 4x LDS (NuPAGE) and 100 mM DTT to a final volume of around 40 µl and boiled at 95 °C for 10 min. After spinning down, each part of each sample was run on a 4-15% Criterion TGX StainFree Protein Gel (26 wells, Bio-Rad, #5678085) in 1x SDS running buffer at 200 V for 32 min. Transfer of proteins to a nitrocellulose membrane (Bio-Rad, #1620112) was performed using the Trans-Blot Turbo Transfer System (Bio-Rad). Following the transfer, western blot was performed as described above. For the second run (Fig. 5a, b), four embryo extracts were prepared, combined and concentrated, as above, to 1 ml. Then half of the sample was treated with Sm nuclease for 30 min at 4 °C, prior to size-exclusion chromatography, while the other half was not.
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