Drosophila Uri, a PP1 alpha binding protein, is essential for viability, maintenance of DNA integrity and normal transcriptional activity

Citation for published version:
Kirchner, J, Vissi, E, Gross, S, Szoor, B, Rudenko, A, Alphey, L & White-Cooper, H 2008, 'Drosophila Uri, a PP1 alpha binding protein, is essential for viability, maintenance of DNA integrity and normal transcriptional activity' BMC Molecular Biology, vol 9, 36, pp. -. DOI: 10.1186/1471-2199-9-36

Digital Object Identifier (DOI):
10.1186/1471-2199-9-36

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher's PDF, also known as Version of record

Published In:
BMC Molecular Biology

Publisher Rights Statement:
RoMEO green

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and/or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.
**Abstract**

**Background:** Protein phosphatase 1 (PP1) is involved in diverse cellular processes, and is targeted to substrates via interaction with many different protein binding partners. PP1 catalytic subunits (PP1c) fall into PP1\(\alpha\) and PP1\(\beta\) subfamilies based on sequence analysis, however very few PP1c binding proteins have been demonstrated to discriminate between PP1\(\alpha\) and PP1\(\beta\).

**Results:** URI (unconventional prefoldin RPBS interactor) is a conserved molecular chaperone implicated in a variety of cellular processes, including the transcriptional response to nutrient signalling and maintenance of DNA integrity. We show that *Drosophila* Uri binds PP1\(\alpha\) with much higher affinity than PP1\(\beta\), and that this ability to discriminate between PP1c forms is conserved to humans. Most Uri is cytoplasmic, however we found some protein associated with active RNAPII on chromatin. We generated a *uri* loss of function allele, and show that *uri* is essential for viability in *Drosophila*. *uri* mutants have transcriptional defects, reduced cell viability and differentiation in the germline, and accumulate DNA damage in their nuclei.

**Conclusion:** Uri is the first PP1\(\alpha\) specific binding protein to be described in *Drosophila*. Uri protein plays a role in transcriptional regulation. Activity of *uri* is required to maintain DNA integrity and cell survival in normal development.

**Background**

Biochemical analysis of protein phosphatase activity led to the identification of distinct enzyme classes based on sensitivity to inhibitors, *in vitro* substrate specificity and cation requirements. Type 1 protein phosphatase (PP1) is one of the major serine/threonine phosphatase classes found in all eukaryotic cells. Cloning of the catalytic subunits of PP1 (PP1c) revealed that there are distinct enzyme forms which had not been distinguished biochemically. Phylogenetic analysis has revealed that there is an evolutionarily conserved distinction between animal PP1\(\alpha\) (human PP1\(\alpha\) and \(\gamma\); *Drosophila* PP1\(\alpha\)\(87B\), PP1\(\alpha\)\(13C\) and PP1\(\alpha\)96A) and PP1\(\beta\) (human PP1\(\beta\) or PP1\(\delta\); *Drosophila* PP1\(\beta\)\(3C\)) implying that the gene products have distinct biological functions despite their identical biochemical properties *in vitro* and >85% sequence iden-
PP1 has numerous diverse functions within the cell including regulation of cell cycle, cytoskeleton, transcription and synaptic plasticity [6-9]. While, in vitro, the catalytic subunit (PP1c) dephosphorylates a wide variety of substrates, the enzyme in vivo is found in a variety of complexes with different protein partners [10]. These PP1 interacting proteins target PP1c to specific subcellular locations, and modulate its activity towards different substrates. When complexed to a regulatory subunit, PP1c becomes much less promiscuous in its activity, so genuine PP1 interacting proteins tend to inhibit PP1c activity in standard in vitro assays (against phosphorylase a or myelin basic protein), even though their in vivo role is to promote PP1c’s activity towards a specific substrate. At least 50 PP1c targeting subunits have now been described, including the G-subunit, that targets PP1c to glycogen particles, the M-subunit that targets PP1c to myosin, and Sara that targets PP1c to the TGFβ receptor [10-13].

The three human PP1c isozymes localise to different subcellular regions in both mitotically active cells and in terminally differentiated cells [14-17]. This suggests that there are targeting subunits that differentiate between the PP1c proteins. So far, mammalian neurabin I and neurabin II/Spinophilin, have been shown to selectively co-precipitate with mammalian PP1γ and PP1α in preference to PP1β [18,19]. Repo-man has a modest (3-fold) preference for PP1γ over PP1α, however the ability of Repo-man to distinguish between PP1γ and PP1β has not been reported [20]. Recent co-immunoprecipitation assays have identified a few more mammalian isozyme specific PP1-interacting proteins [21]. In Drosophila, one PP1β-specific binding protein has been described, MYPT-75D; this probably is important for mediating the single essential function of PP1β in flies, which is regulation of non-muscle myosin [3]. No Drosophila proteins with a preference for binding PP1α rather than PP1β have been described.

URI (unconventional prefoldin RPB5 interactor) has been implicated in modulation of the transcriptional response to nutritional cues in humans and S. cerevisiae [22]. URI mutant S. cerevisiae are viable, but constitutively over-express genes important for amino acid metabolism. C. elegans uri-1 mutant animals are also viable, but have defects in germ cell proliferation and DNA stability [23]. Human RMP (RPB5-mediating protein) is identical to URI, except that the clone described lacks the N-terminal 25aa. RMP was identified through its ability to bind the RPB5 subunit of RNA polymerase, and was demonstrated to have weak transcriptional co-repressor activity [24,25]. URI (lacking the first 75aa) has also been named NNX3 [26]. Here we present a functional analysis of the Drosophila uri gene.

Results

**URI is a PP1α specific binding protein**

To identify potential regulatory subunits of the major protein phosphatase catalytic subunit of Drosophila we screened a yeast two-hybrid library using Drosophila PP1α87B as a bait and isolated 25 cDNAs representing 16 different genes. One of these genes (CG11416, uri) was represented by 2 independent clones. CG11416 has been described as the Drosophila homologue of URI since the N-terminal region (aa 30–124, wavy box in Figure 1B) contains a Prefoldin domain, most similar to that of human URI (RMP, NNX3), and the C-terminus contains a short region of homology termed the URI-Box (aa 720–729, grey box in Figure 1B) (Full alignment shown in [22], supplementary material). The uri predicted transcript encodes a protein of 731 amino acids with a calculated molecular weight of 84 kDa, although the protein runs at 110 kDa in SDS PAGE, and an isoelectric point (pI) of 4.66. Two coiled coil domains outside the prefoldin domain are predicted (striped boxes in Figure 1B). Human URI does not contain additional coiled-coil regions, but C. elegans uri-1 does. The overall acidity of the protein is partly explained by a very acidic region at aa 170–185. Human URI also has an acidic domain, as does worm uri-1. Drosophila URI has three putative PP1c binding motifs ([KR][0,1][V][I][X [FW]]) at amino acids 337 (KVNF), 403 (RISF) and 469 (RNIEF), (asterisks in Figure 1B), while human URI and worm uri-1 each contain one PP1c binding motif (RVEF in human; KIKF in worm) at the end of the prefoldin domain. Drosophila Uri contains four predicted nuclear localisation signals (NLS) (inverted triangles in figure 1B); both human URI and worm uri-1 have two NLSs. The C-terminal region of Drosophila Uri contains a repeat sequence (from aa 504–568 and aa 587–651, stippled in Figure 1B) that shows no homology to the URI proteins from other species, or to any other protein sequence in the database (NCBI Blast).

The Drosophila PP1c genes encode proteins that are >85% identical to each other and have indistinguishable activities in vitro. Nevertheless, PP1β9C is structurally distinct from the PP1α isozymes and is encoded by an essential gene [1], suggesting some binding partners can distinguish between PP1c isozymes. Although uri was isolated in our small scale PP1α87B yeast two-hybrid screen, no uri clones were isolated in a 10-fold larger scale screen from the same library using PP1β9C as a bait [12], suggesting that Uri may be a PP1α-specific binding protein. We directly tested this in yeast two-hybrid, and found that
Uri was able to bind all three PP1α forms, but was unable to interact with PP1β9C (Figure 2A). PP1c dephosphorylates a wide range of substrates in vitro; substrate specificity in vivo is provided by targeting subunits. Addition of a PP1 targeting subunit will typically alter substrate specificity in the in vitro reaction, and therefore inhibit the ability of PP1c to dephosphorylate a wide variety of phosphosubstrates. We tested the ability of Uri to inhibit the myelin basic protein (MBP) phosphatase activities of recombinant PP1α87B and PP1β9C. Bacterially expressed Uri inhibited the PP1α87B MBP phosphatase activity with higher affinity than the PP1β9C MBP phosphatase activity. The Uri IC_{50} against PP1α87B was about 25-fold lower than that of Uri against PP1β9C (Figure 2B). No antibodies have been produced that can distinguish between the Drosophila PP1 proteins, so, to test the interaction between Uri and PP1α and PP1β in vivo, we ectopically expressed HA-tagged PP1α87B and PP1β9C proteins in flies, and tested their ability to co-immunoprecipitate with endogenous Uri. arm-GAL4 [29] flies have low-level ubiquitous expression of the yeast transcription factor Gal4p, which activates expression of transgenes under the control of the Gal4p target site, UAS. Immunoprecipitation of cell lysates of arm-GAL4; UAS-HA-PP1α87B and arm-GAL4; UAS-HA-PP1β9C flies with anti-Uri antibodies, followed by immunoblotting with anti-HA antibodies, showed that HA-PP1α87B co-precipitated with Uri, but HA-PP1β9C did not. This indicates that Uri can interact with PP1α, but not with PP1β in vivo.
iterated more efficiently with Uri than did HA-PP1β9C (Figure 2C), consistent with PP1c activity assay data. The reciprocal experiment, immuno-precipitation with anti-Uri antibodies and immunoblotting with anti-Uri antibodies confirmed this difference in the strength of the interactions (data not shown). Therefore Uri is the first

**Figure 2**

**Uri is a PP1α specific binding protein.** A. Uri binds all three Drosophila PP1α isoforms (PP1α13C, PP1α87B and PP1α96A) in yeast two-hybrid assays, but does not bind Drosophila PP1β (PP1β9C) in this assay. NIPP1 shows no discrimination between the isozymes, and is shown as a control. PP1 isoforms were expressed as DNA binding domain fusions (bait), while Uri and NIPP1 were Activation domain fusions (prey). B. Uri is an specific inhibitor of Drosophila PP1. The myelin basic protein phosphatase activity was measure in the presence of different concentrations of recombinant PP1α87B (triangles) or PP1β9C (squares). Phosphatase activity is shown as % of control in the absence of Uri. C. Uri immunoprecipitates ectopically expressed HA-PP1α87B more efficiently than HA-PP1β9C from fly extracts. Western blot showing similar levels of expression of HA-PP1α87B and HA-PP1β9C in total fly lysate, and proportion immuno-precipitated with the anti-Uri antibody. Normal guinea pig serum and Protein G sepharose only controls show no precipitation of the expressed proteins. D. Human PP1α (first lane) but not PP1β (second lane) co-immunoprecipitates with URI/RMP, and not URI/RMP-D2 (lanes 3 and 4) when co-expressed in COS7 cells (first panel). Expression controls are shown in the second and third panels.
Drosophila PP1c interacting protein to be shown to interact with PP1α with significantly higher affinity than with PP1β.

Since human URI also contains a predicted PP1c binding motif we investigated its ability bind different human PP1c isoenzymes. FLAG-tagged URI/RMP was transiently expressed in COS 7 tissue culture cells along with V5-tagged human PP1α or PP1β; PP1α was expressed more strongly in these experiments than PP1β. Immunoprecipitation of cell lysates with anti-FLAG antibodies followed by immunoblotting with anti-V5 antibodies showed that human PP1α co-precipitated efficiently with the human URI. Co-immunoprecipitation of PP1β and FLAG-tagged URI/RMP was not detected (Figure 2C). A deletion construct of the human URI/RMP (RMP-D2) has been reported [25], the region of URI/RMP included in this construct is indicated by the bracket below the human protein in Figure 1. The putative PP1 binding sites in URI/RMP are missing in this deletion derivative. We found no co-immunoprecipitation between either of the PP1 isoenzymes and URI/RMP-D2. This consistent with the notion that the RVEF putative PP1 binding site on URI is important for the URI-PP1c association, although other sites missing in the truncated protein could also be implicated in the interaction. Drosophila Uri could also bind mammalian PP1α with higher affinity then PP1β when they were co-expressed in mammalian tissue culture (data not shown). Therefore, despite low overall sequence homology, the ability of Uri to discriminate between different PP1c isoenzymes is evolutionarily conserved.

**Uri protein is predominantly cytoplasmic**

Human URI is an RPB5 binding protein [25]. This interaction, along with the predicted nuclear localisation sequence and the known role for human URI in transcriptional regulation, would predict that at least some URI protein should be nuclear and chromatin associated. To test this, we transiently expressed FLAG-tagged Drosophila Uri, human URI/RMP and URI/RMP-D2 and visualised their localisation by immunofluorescence. URI/RMP-D2 lacks the prefoldin domain but retains the ability to bind RPB5 in COS7 mammalian tissue culture cells [25]. We found that Uri (Drosophila) and URI/RMP (human) proteins were predominantly cytoplasmic with perinuclear localisation (Figure 3A, B). This is consistent with the cytoplasmic localisation for the N-terminally deleted NNX3 clone of URI/RMP [26]. When URI/RMP-D2 was expressed, strong nuclear localisation of the protein was observed (Figure 3C), indicating that the regions deleted from this construct are important for regulation of the nuclear localisation of URI protein. Human URI/RMP has been shown to bind DMAP1, a DNA methyltransferase-1 associated protein implicated in gene silencing, and this interaction promotes the nuclear re-localisation of URI/RMP [24]. To test whether the interaction between Uri and PP1 similarly altered the subcellular localisation of either protein, we co-expressed Drosophila Uri with PP1α87B in COS7 cells. PP1α87B, when expressed alone, can be detected in the cytoplasm, but is primarily nuclear (single transfected cell indicated by an arrow in Figure 3E). When PP1α87B was co-expressed with Uri, both proteins were more abundant in the cytoplasm; both the nuclear accumulation of PP1α87B and the perinuclear accumulation of Uri was lost (Figure 3D–F, arrowhead indicates nucleus of a co-transfected cell).

**uri is expressed throughout development, but is most abundant during embryogenesis, pupariation, and in adult gonads**

We examined the developmental protein expression profile by Western blotting and found Uri protein to be most abundant in early embryos and pupae. The protein could not be detected in extracts of whole adult flies, or adults lacking gonads, however Uri was detected in extracts of ovaries and testes (Figure 4A). To determine the cellular and tissue distribution of uri transcription we used RNA in situ hybridisation. uri mRNA expression was uniform in embryos, imaginal discs, and larval brains (data not shown). In testes, uri was expressed in mitotically proliferating spermatogonia and in early primary spermatocytes, with staining levels decreasing as spermatocytes matured (Figure 4B). No transcripts were detected in post-meiotic stages. Male germline stem cells may express low levels of the mRNA.

**Uri protein is in cytoplasmic speckles in vivo**

Examination of protein subcellular localisations after ectopic expression can be complicated by artefacts associated with saturating the normal localisation machinery. Therefore we examined the subcellular distribution of endogenous Drosophila Uri, using the anti-Uri antibody, in tissues in which we know from Western blotting there are significant levels of Uri protein. In wild type primary spermatocytes (all stages), and maturing spermatids (not shown) Uri was found throughout the cytoplasm, with a distinctive concentration in small speckles (Figure 5A–C). No specific localisation to the nucleus was apparent. Persistence of Uri into post-meiotic stages indicates that it has a long half life, as no transcript was detected at this stage. To test whether the localisation in testis is simply an oddity of this tissue we examined Uri localisation in salivary glands and embryos. In salivary gland Uri was predominantly cytoplasmic, with a mild perinuclear accumulation (Figure 5D–F). In embryos we also found that Uri protein in interphase cells is primarily cytoplasmic, and some protein was in speckles in both the nucleus and the cytoplasm. The speckles and uniform staining persisted in mitotic cells, and no localisation to condensed chromosomes was found (Figure 5G–I).
Uri protein is associated with transcriptionally active regions of polytene chromosomes

Although the majority of endogenous Drosophila Uri protein is cytoplasmic, the published interactions between Uri homologues and RNA polymerase II suggested that at least some Uri protein would be associated with chromatin. We examined the localisation of Uri on spreads of larval salivary gland polytene chromosomes and found that Uri stains numerous discrete bands. Only chromatin associated proteins are preserved for staining in these spread preparations, explaining the discrepancy between the chromatin localisation seen in spreads and the cytoplasmic localisation seen in whole mount. Co-labelling with an antibody recognising active RNA polymerase II revealed that the majority of the Uri positive bands are sites of active transcription (Figure 6A–C). Drosophila larvae, when stressed by heat shock, shut off most transcription and only actively transcribe from the heat shock response loci. This is associated with re-localisation of RNA polymerase II to a small number of heat-shock induced puffs on the polytene chromosomes [30]. We found that Uri also relocated to the heat shock puffs, and was lost from the remainder of the polytene chromosomes, on heat shock treatment (Figure 6D–F). Therefore, although most Uri protein is cytoplasmic in salivary gland cells, some is nuclear, and associated on chromatin with sites of active transcription.

uri is essential for viability in Drosophila

Mutation of C. elegans uri-1 leads to sterility as well as multiple and variable somatic defects [23]. The budding yeast URI deletion strain is viable, but defective for expres-
The uri\textsuperscript{i} chromosome was homozygous lethal, however uri\textsuperscript{i}/Df(2R)Px2 males and females were viable, fertile, and did not exhibit a detectable phenotype (Df(2R)Px2 deletes the uri locus). Thus the lethality of the uri\textsuperscript{i} chromosome was due to one or more second-site lethal mutations. P-element insertions in promoters or 5' UTRs often down-regulate transcription of the respective gene. Using RT-PCR designed against a region 3' of the uri\textsuperscript{i} insertion, we found that there was considerably less uri transcript in uri\textsuperscript{i}/uri\textsuperscript{i} compared to uri\textsuperscript{i}/CyO, act-GFP first instar larvae, showing that the P element in uri does indeed down-regulate uri transcription (data not shown). To generate stronger loss of function mutant alleles, we screened for deletions caused by imprecise excision of the uri\textsuperscript{P} element and found nine that specifically disrupted uri. These deletions varied in size from 0.6 to 1.7 kb. We selected uri\textsuperscript{i10b} for further analysis as it has the largest deletion, removing the translation start, exon 2 and most of exon 3 (Figure 1A). The second site lethal mutation from the original chromosome was separated from the uri\textsuperscript{i10b} allele by meiotic recombination. Although uri\textsuperscript{i10b} deletes a significant proportion of the uri gene, we found that a transcript annealing most of the 5' UTR to the final 634 bp of the wild type transcript is expressed in uri\textsuperscript{i10b} mutant embryos. Two possible peptides encoded by this mutant transcript are shown (Figure 1B).

**uri is required for full expression of some genes in embryos**

uri\textsuperscript{i10b} homozygote embryos hatch normally, as do uri\textsuperscript{i10b}/Df(2R)Px2 embryos. The mutant first instar larvae appeared sick, for example showing very little locomotion or feeding activity, and died soon after hatching. To determine whether this lethality could be attributed to defects in transcriptional activity we used RT-PCR to compare expression levels of several genes in mutant vs heterozygous sibling embryos. We chose to analyse expression of ebony, CG3999 and CG1315, the *Drosophila* homologues of *S. cerevisiae* LYS2, GCV2 and ARG1 respectively, which were shown to be regulated by scUri [22]. Expression of *ebony* and CG1315 was significantly and reproducibly lower in mutant embryos than wild type embryos, while expression of CG3999 was slightly elevated, or not altered in the mutant background (Figure 6C). Selection of a control gene in these experiments is not straightforward, standard choices such as a ribosomal protein are not necessarily appropriate when signalling downstream of TOR, which regulates growth and metabolism, could be affected. As a control we chose PP1\betaC, which we had no reason to expect to change. Over several experiments expression of PP1\betaC was somewhat variable between in mutant vs wild type, but there was never more than a 2-fold difference in expression of this gene in the two conditions. *Drosophila* uri is therefore essential for normal expression of at least two (probably more) target genes in
Figure 5

**Uri is predominantly cytoplasmic, and has a speckled distribution pattern in testes and embryos.** Immunofluorescence showing Uri localisation (A, green in C) to cytoplasmic speckles in wild type primary spermatocytes (A-C). The nuclear envelopes are labelled with anti-lamin antibodies (B, red in C). D-F, Salivary gland cells stained for Uri (D, green in merge) and DNA (E, red in merge) revealing that Uri is predominantly cytoplasmic, and is concentrated in the perinuclear region. G-I, Uri (G, green in I) is also localised to cytoplasmic speckles in interphase cells of cellularised early embryos. Cellular structure and mitotic regions were revealed by anti-tubulin staining (H, red in I). In cycle 14 mitotic domains (upper left region of figure) the Uri staining remained speckled, but was distributed uniformly in the cells.
Figure 6

Uri is on active chromatin in polytene chromosome spreads. A-F, Polytene chromosomes from wild type larvae stained for Uri (green), active RNAPII (red) and DNA (blue). Higher power single channel greyscale images of the boxed region are shown in A, B, and the merge of these is shown in C. Uri co-localises with active RNAPII on normal larval polytene chromosomes (A-C; overlap is yellow in C). D-F, After heat shock, RNAPII activity is restricted to the heat-shock puffs (E), Uri co-localises to these puffs (D, F). G, RT-PCR of potential uri target genes from uri110b homozygous embryos and uri110b/Cyo GFP sibling control embryos. The results of three independent experiments are shown; size marker is shown on the left. mRNA levels of CG3999 and PP1β9C varied somewhat between experiments; CG3999 was slightly reduced in mutant compared to control embryos, PP1β9C on average was equal in mutant compared to control. CG1315 and ebony mRNA levels were significantly and reproducibly lower in the mutant embryos than in control embryos. GFP control primers confirm the accuracy of embryo selection based on fluorescence. Asterisks indicate primer dimer bands.
embryos, but is not required for the expression of all genes.

**uri is required for cell viability in the germline**

Expression of His-Myc tagged Uri was able to partially rescue the uri^{110b}/Df(2R)Px2 lethality, so that flies of the genotype UAS-HM-uri, uri^{110b}/arm-GAL4, Df(2R)Px2 were mostly late pupal lethal, with approximately 2% adult survivors. The surviving adults had weak locomotory ability and activity, and died within 24 hours. Nearly all of them exhibited additional wing vein material along the wing veins L2, L4, and L5; some pharate adults had small eyes. We found that expression of a hairpin uri RNAi construct in the eye imaginal disc gave a similar small eye phenotype (data not shown). We confirmed the absence of full length Uri protein in uri^{110b} with Western blotting on wing disc samples from UAS-HM-uri, uri^{110b}/arm-GAL4, Df(2R)Px2 third instar larvae (Figure 5C).

Uri protein is apparently gonad specific in adults (Figure 4A), therefore we wanted to examine the mutant phenotype in ovaries and testes. UAS-HM-uri is a P[UAST] derivative which does not express in the female germ-line [32], while arm-GAL4 does not express efficiently in the male germline. This lack of germline expression means that the UAS-HM-uri, uri^{110b}/arm-GAL4, Df(2R)Px2 are essentially only rescued in the soma, allowing us to analyse the requirement for uri in the germline. These animals are developmentally delayed compared to wild-type, so their gonads had later stages of spermatogenesis or oogenesis than age matched controls.

Testes from uri somatically-rescued males were much smaller than their wild type counterparts (compare Figures 7A to 7B, and 7D to 7E). The testes contained a few apparently normal spermatogonia and spermatocytes (Figure 7E, arrow) as well as some elongated spermatids. Post-meiotic spermatids usually had abnormal morphology, although some testes contained a small number of normal motile sperm (Figure 7B, arrow). In addition to the healthy cells, mutant testes were full of dying cells and debris from dead cells (Figure 7E, asterisk). We were able to partially rescue the testis phenotype of somatically rescued uri^{110b}/Df(2R)Px2 mutants by additional expression of uri in late spermatogonia and spermatocytes using the 8am-GAL4-VP16 driver [33]. These pheratite adult males had longer testes than those lacking the germline expression (Figure 7C); the testes contained many later stages of spermiogenesis, and numerous motile sperm (arrow in Figure 7C). They were however not fully rescued, as they contained only a few cysts of spermatogonia and spermatocytes, and post-meiotic spermatids were located much closer to the apical tip of the testis than is normal (Figure 7F, asterisk, compare to 7D).

The ovarioles in ovaries of wild type pharate adult females typically have a germarium and three egg chambers, the oldest of which is at about stage 5 of oogenesis. Ovaries from uri somatically-rescued pharate adult females were small, and the germaria of these ovaries were thinner than wild type (data not shown). Most ovarioles lacked well defined stage 1 and later egg chambers, although one or two apparently normal later stage (up to stage 7) egg chambers were present in most ovaries.

**uri mutant cells contain damaged DNA**

C. elegans uri-1 mutants, while viable, were sterile due to germine proliferation failures caused by loss of DNA integrity. To test whether the *Drosophila* *uri* gene also has a role in DNA maintenance we examined testes of somatically rescued uri^{110b} male larvae using TUNEL labelling. As a positive control we treated wild type testes samples with DNase to induce DNA breaks, while untreated wild type testes served as a negative control. We observed high levels of TUNEL labelling of DNA in *uri* somatically rescued testes, indicating that these cells contained damaged DNA (Figure 8A, B). The most mature primary spermatocytes showed lower levels of TUNEL labelling. Negative control testes showed only background TUNEL labelling in the cytoplasm (Figure 8C, D). We also saw elevated levels of TUNEL labelling in somatic tissues, for example in fat body, from these larvae, consistent with their low viability to adulthood (data not shown).

**Discussion**

*S. cerevisiae* scUri (BUD27) mutants have defects in bud site selection and in the transcriptional response downstream of TOR (target of rapamycin) signalling [22,34]. siRNA mediated depletion of *URI* also led to TOR response defects in human tissue culture cells [22]. *C. elegans* *uri-1* mutants, while viable, had pleiotropic developmental defects and were sterile due to germine proliferation failures caused by loss of DNA integrity [23]. These apparently unconnected phenotypes suggest that *uri* is involved in multiple processes, making a strong case for its further characterisation.

Numerous lines of evidence implicate *uri* in transcriptional regulation. Both human and yeast URI proteins function as weak transcriptional repressors; scURI is also a context dependent activator [22,24,25]. Yeast URI (Bud27p) also binds to Gis1p’s jmjC (histone demethylase) domain [35]. Human RMP (URI) was identified as a binding partner of RBP5, an RNA polymerase subunit. Consistent with this, we found that a fraction of endogenous Uri protein is associated with sites of active transcription on salivary polytene chromosomes and this association was maintained as the transcriptional profile changed in response to heat shock. This is consistent with
uri is required for male germline viability and differentiation. A, wild type testis from male pharate adult. Stem cells reside at the apical tip (asterisk), cells move distally as they mature so most of the apical region is taken up by maturing primary spermatocytes. Elongating spermatids are seen pushing up the length of the testis. No motile sperm have yet developed. B, somatically rescued uri^110b/Df testis from male pharate adult (UAS-HM-uri, uri^110b/arm-GAL4, Df(2R)Px2), shown at the same scale as A. Some healthy spermatocytes near the apical tip (asterisk) and elongating stages are visible, as well as one motile sperm (arrow). Most of the testis is filled with degenerating dead cells. C, Testis from uri^110b/Df male pharate adult rescued in the soma and germline from late spermatogonia (UAS-HM-uri, uri^110b/arm-GAL4, Df(2R)Px2; Bam-GAL4-VP16/+), same magnification as A and B. The testis is significantly larger than without germline rescue, but smaller than wild type. Few early stage cells are seen (apical region marked with asterisk), but there are many differentiating spermatid bundles. Numerous motile sperm are visible (arrow). D, higher power view of apical region of wild type pharate adult testis. E, apical region of somatically rescued pharate adult uri^110b/Df testis (genotype as B), same magnification as D. Healthy primary spermatocytes are indicated by the arrowhead, dead cells by the asterisk. F, apical region of uri^110b/Df testis (genotype as C) from a pharate adult rescued in the soma and germline from late spermatogonia, same magnification as D and E. Fewer small cells than in wild type are present in the apical region, and post-meiotic spermatids (asterisk) are much closer to the apical tip.
**Figure 8**

*uri* mutant cells accumulate DNA damage. A, TUNEL labelling and B, DNA labelling, of a testis from a somatically-rescued *uri* mutant larva. Strong TUNEL staining was found on the DNA of spermatogonia (large arrow) and early primary spermatocytes (small arrow). Lower levels of TUNEL staining are seen in the oldest cysts present in these testes (mature primary spermatocytes, arrowhead). C, D, wild type control larval testes show only background cytoplasmic TUNEL staining. E, F, TUNEL staining is seen on DNA of wild type larval testis cells after treatment with DNAse I (positive control). The large nucleus in this figure (arrow) is from the fat body.
a role for Uri in general transcriptional activation rather than repression.

Given the chromatin functions of Uri-containing complexes it is surprising that endogenous Uri is predominantly cytoplasmic. The URI/RMP prefoldin domain C-terminal half, with a predicted coiled-coil structure, acts as a cytoplasmic anchor in human cells [24]. This region interacts with DNA methyltransferase 1-associating protein, and this interaction promotes nuclear re-localisation. The putative PP1c interacting motif of the human protein also resides at the prefoldin domain C-terminus. Over-expression of Drosophila Uri prevented nuclear accumulation of co-expressed PP1α, indicating that PP1 does not promote Uri nuclear localisation (Fig 4). When ectopically expressed in human tissue culture cells, Drosophila Uri and human Uri showed a perinuclear accumulation, this was also seen in salivary glands in vivo. In other cells, most obviously spermatocytes, Uri localised to cytoplasmic speckles. We are unsure what organelle or subcellular structures are associated with Uri speckles, although the speckles do not co-localise with the Golgi apparatus, or with P-bodies, which have similar speckled cytoplasmic localisation patterns in spermatocytes (data not shown).

Since both ScUri and human URI have been implicated in TOR signalling we examined expression in uri null embryos of Drosophila homologues of genes downstream of scUri. Two of the genes tested, CG1315 and ebonymbreductively expressed at lower levels in mutant embryos. Thus, in contrast to the yeast situation, uri is required for full expression of these genes. TOR signalling in Drosophila is important for larval growth; mutant larvae grow slowly, but live for up to 30 days [36]. uri1106 larvae die soon after hatching and are not developmentally delayed, indicating that, although uri may be implicated in regulation of TOR target genes, it probably has a wider range of target genes and/or other cellular functions. Further examination of the transcript profiles in mutant animals, for example by microarray analysis, would reveal the full extent of the transcriptional defect in uri mutant larvae. Human URI was isolated in a complex that also contained TIP48, TIP49, RBP5 and several small prefoldin domain proteins [22]. Like uri, Drosophila TIP48 (reptin) and TIP49 (pontin) mutant die as first instar larvae with no obvious defects [37], so uri lethality could potentially be attributable to defects in a complex containing these ATPases.

Uri protein is most abundant in embryos, pupae and in adult gonads; where expression is higher in germline than soma. This germline enrichment of fly Uri correlated well with the sterility phenotype in worms, and led us to investigate uri’s role in gonads in more detail. Partial somatic rescue of uri mutant flies enabled us to analyse the cell autonomous germline role, and revealed strong effects in both males and females. In both sexes the major defect was reduced cell viability. Spermatogenesis in flies is maintained by a population of stem cells, which give rise to spermatogonia. Defects in stem cell self renewal or survival eventually lead to empty (or nearly empty) testes, as stem cells are not maintained. Similarly, defects in spermatogonial survival lead to extremely small testes, as only stem cells remain. The somatically rescued uri male phenotype is consistent with loss of stem or early spermatogonial cells. The testes were mostly filled with dead or dying cells, indicating that uri is required for cell viability. Very few later spermatids were found, although we could occasionally see motile sperm. These would have initiated spermatogenesis in early larvae, and may have been saved by perdurance of maternally provided Uri protein. Provision of Uri to the germline using Bam-Gal4-VP16, which expresses in late spermatogonia and early spermatocytes, partially rescued the testis phenotype. Many more later stages of spermatogenesis were seen, indicating that uri is required for the viability of late spermatogonia and spermatocytes. Testes rescued by expression of uri with Bam-Gal4-VP16 had fewer early spermatogonia than wild type testes. The inefficient rescue of these cells show that uri is required in all spermatogonia, and possibly also in germ line stem cells, to maintain cell viability. The RNA in situ confirms that uri is most highly expressed in spermatogonia and early spermatocytes. Somatically rescued uri females had thinner germaria than wild type, and mostly lacked later oogenesis stages.

C. elegans uri-1 is important to maintain DNA stability in the worm germline. By analogy with worms, the cell death could be due to accumulation of DNA damage; it could also be due to transcriptional defects, as uri is required for transcription. We established that uri mutants have defects in maintenance of DNA integrity, as shown by TUNEL staining. This is in complete agreement with the C. elegans findings. However, we cannot rule out the possibility that primary defects in transcriptional regulation lead to the DNA damage phenotype as a secondary effect.

Most PP1c interacting proteins do not discriminate between PP1α and PP1β isozymes. MYPT-75D was the first Drosophila protein to show differential binding, having higher affinity to PP1β than PP1α. This specificity is linked to the essential role of PP1β in flies – non-muscle myosin regulation [3]. Uri is the first Drosophila protein to be demonstrated to have a strong preference for PP1α over PP1β. An essential, non-redundant function for PP1α is suggested by inability of PP1β to rescue PP1α mutants [5]. Lethality of uri mutants supports the notion that PP1α has a role that cannot be supplied by PP1β. Uri can bind all the Drosophila PP1α isozymes, and indeed was identified as a PP1α96A putative interacting protein in a large scale yeast two-hybrid screen [38]. Canonical
PP1c binding sites in human and worm URI suggested that binding to PP1 was probably conserved for this protein. We confirmed the human URI-PP1c interaction directly, adding URI to the ever-growing list of PP1c binding proteins. More excitingly, we show that interaction with PP1α in preference to PP1β is conserved between fly and mammalian URI.

**Conclusion**

Here we have shown that *Drosophila uri*, is an essential PP1α-specific binding protein. Using genetic and biochemical analyses we implicate *uri* in regulation of transcription, germ-line and somatic cell viability and maintenance of DNA integrity.

**Methods**

*Drosophila culture and strains*

*Drosophila* were cultured on standard yeast/glucose/maize (or wheat flour) media, at 25°C. Wild-type was OregonR. *P[Gsv6]* was generated by DGSP [31] and kindly provided by Toshiro Aigaki (Tokyo Metropolitan University, Japan). *Bam-Gal4-VP16* was provided by Dennis McKearin [33]. Other lines used were obtained from Bloomington *Drosophila* stock centre, and are described in Flybase [39]. *UAS-HM-uri* transformants were selected by standard techniques after injection of pP{UAS-HM-uri} into w1118 embryos. The *uri* deletion was made by selection of *w* excisions of the *P[Gsv6]* *w* element using CyO, Δ2-3 as a transposase source. In total 509 excision lines were generated, of which 31 were lethal in trans to *Df(2R)Px2*. These were tested by PCR to identify which genes in the region were affected (CG11414, *uri* or CG12252).

**Yeast two-hybrid screen**

A two-hybrid *Drosophila* third instar larval cDNA library constructed in pACT [40] was screened in the yeast strain Y190 using a full length PP1α87B cDNA fused to GAL4 (pAS2-PP1α87B) as a “bait”, as described in [41]. Two independent clones of *uri* (CG11416) were isolated.

**Molecular cloning and plasmid construction**

A full-length *uri* cDNA was created from the longest partial EST available at the time (LD39507) whose 5’ end is 13 bp downstream of the *uri* ATG initiation start codon. The cDNA was amplified by PCR using Platinum Pfx DNA Polymerase (GIBCO BRL) with the missing sequence, to the ATG incorporated within the 5’ primer. This yielded a 2437 bp PCR product that was directionally cloned into *NdeI/NotI* sites of pGBK7 for yeast two-hybrid screening. This *uri* fragment was subcloned into FLAG-pcDEF3 for mammalian tissue culture expression, into pET-28m for bacterial expression, and into pP{UAS-HM-uri} [42] to create pP{UAS-HM-uri} for *Drosophila* transgenesis. The pAS2-PP1c constructs used contain full length PP1c (PP1α87B, PP1α96A, PP1α13C or PP1β9C) fused to the DNA binding domain of GAL4 [41]. A partial cDNA clone of the *Drosophila* homologue of NIPP1 (NIPP1Dm) fused to the activation domain of GAL4 in pACT served as a positive control for two-hybrid interactions. pNKFlag-RMP and pNKFlag-RMP-D2 mammalian expression constructs were kindly provided by Seishi Murakami (Kanazawa University, Japan). Mammalian tissue culture expression constructs of human PP1α (clone H-X70848M) and human PP1β (clone H-X80910M) in the vector pcDNA3.1/GS were from Invitrogen. The Rpb5 ORF was amplified by PCR from *Drosophila* genomic DNA and subcloned into the PET28a bacterial expression vector (Novagen).

**Preparation of protein extracts from flies**

Adult females were collected and either stored at -80°C or used immediately. Flies were homogenized at 4°C in IP buffer (25 mM HEPES, pH 7.5, 100 mM NaCl, 1 mM EGTA, 0.1% Triton X-100, 10% glycerol containing EDTA-free protease inhibitor mix (Roche, Indianapolis)). Homogenates were clarified by centrifugation (20 min at 10000 × g, 4°C) and the supernatants used in immunoprecipitation and pulldown experiments.

**Western Blotting**

For developmental western blotting, embryos, larvae, pupae and flies were collected, frozen, homogenised in 2 × SDS sample buffer and boiled for 10 min. Samples were stored at -20°C and spun again before loading. For wing disc samples, 20 wing imaginal discs were taken up in 10 mM Tris-HCl pH 6.8, 180 mM KCl, 50 mM NaF, 1 mM NaVO4, 10 mM β-glycerolphosphate, 1% Triton X-100, 0.1% Tween 20 and stored at -80°C. An equal volume of 2 × SDS sample buffer was added before boiling and loading. Protein extracts were run on 10% SDS polyacrylamide gels, and transferred to Immobilon-P PVDF nylon membrane. Western blots were stained with Ponceau S, washed and blocked with 5% non-fat dried milk and then probed sequentially with the primary and HRP conjugated secondary antibodies (Sigma). Detection was by Supersignal chemi-luminescence (Pierce).

**Preparation of recombinant proteins and phosphatase assays**

Recombinant NH2-terminal His6-tagged PP1β9C, PP1α87B, (cloned into pET28a) and Uri (pET28m) were expressed in *E. coli* BLR21 (DE3) cells. His6-tagged proteins were purified using NiNTA agarose (Qiagen) following the manufacturer’s instructions. Renaturation of PP1c was carried out as described in [43]. Recombinant PP1β9C was expressed in *P. pastoris* as described in [44] and was used for the myelin basic protein phosphatase (MYBPP) assays. The myelin basic protein phosphatase
(MYBPP) assays were performed using a Protein Serine/Threonine Phosphatase (PSP) Assay System (New England Biolab) and [\(^{32}\)P] ATP (5000 Ci/m mole from Amer sham Pharmacia Biotech). One unit of MYBP phosphatase is defined as the amount of enzyme which releases 1 nanomole of [\(^{32}\)P] phosphate/minute from [\(^{32}\)P] labelled MYBP in the standard assay.

Transient transfection of COS7 mammalian cells was achieved using FuGENE 6 transfection reagent (Boehringer Mannheim) according to the manufacturer’s instructions. Recombinant proteins were detected by indirect immunofluorescence and confocal microscopy.

**Antibodies**

Anti-Uri polyclonal antibodies were generated by immunising guinea pigs with recombinant full length bacterially-expressed Uri protein (Moravian-Biotechnology). Guinea pig anti-Uri antibody was used at a dilution 1:500–1000 for Western blotting and 1:100 for immunofluorescence (1:20 for polytene chromosome). Anti-lamin antibody (T47, monoclonal supernatant) was kindly provided by D. Glover (Cambridge University, UK) and was used at 1:20–50 dilution for immunofluorescence. Anti-RMP antibody was used for Western blotting at 1:2000 dilution and was kindly provided by Seishi Murakami (Kanazawa University, Japan). Anti-RNAPII H14 (Covance/BabCo) was used at 1:500 dilution for Western blotting and at 1:100 for immunofluorescence. Anti-V5 antibody (Invitrogen) was used at a dilution 1:1000 for Western blotting. Anti-Arm was used at 1:300 (DSHB, Iowa). Secondary antibodies coupled to HRP were diluted 1:1000 for Western blotting. Anti-Uri polyclonal antibodies were generated by immunising guinea pigs with recombinant full length bacterially-expressed Uri protein (Moravian-Biotechnology). Guinea pig anti-Uri antibody was used at a dilution 1:500–1000 for Western blotting and 1:100 for immunofluorescence (1:20 for polytene chromosome). Anti-lamin antibody (T47, monoclonal supernatant) was kindly provided by D. Glover (Cambridge University, UK) and was used at 1:20–50 dilution for immunofluorescence. Anti-RMP antibody was used for Western blotting at 1:2000 dilution and was kindly provided by Seishi Murakami (Kanazawa University, Japan). Anti-RNAPII H14 (Covance/BabCo) was used at 1:500 dilution for Western blotting and at 1:100 for immunofluorescence. Anti-V5 antibody (Invitrogen) was used at a dilution 1:1000 for Western blotting. Anti-Arm was used at 1:300 (DSHB, Iowa). Secondary antibodies coupled to HRP were diluted 1:10000 for Western blotting.

**Immunoprecipitation from flies**

Lysates were pre-cleared by addition of 50 µl of Protein G Sepharose resin (Pharmacia). Following a 15 minute incubation on ice this mixture was centrifuged at 4°C for 1 minute 2500 × g. Aliquots of cleared lysates with 250 µg total protein content were withdrawn and used in IP experiments. After incubation of cleared lysates with the antibody required, 50 µl of equilibrated Protein G Sepharose was added and incubated for 1 hour on ice. Then resin was pelleted as before and washed 3 times with ice cold IP buffer. The beads were finally separated by centrifugation at 4°C 20 min at 10 000 × g, resuspended in 2 × SDS sample buffer and subjected to Western blotting.

**Immunoprecipitation from COS7 cells**

Transfected COS7 cells were washed twice in PBS and lysed in buffer containing 50 mM Tris-HCl pH (7.4–8.0), 0.5% Triton-X100, 150 mM NaCl, protease inhibitors (Roche). After centrifugation, supernatants were incubated with 1–2 µg antibody for 1–3 h on ice with gentle agitation. Equilibrated protein G-Sepharose was added and incubated for 1 hour, pelleted and washed with lysis buffer. Beads were resuspended in 2 × SDS-PAGE sample buffer and subjected to Western blotting.

**Immunofluorescence and in situ hybridisation**

For tissue culture immunofluorescence, mammalian COS7 cells were grown on coverslips, transfected as described above, fixed with 4% paraformaldehyde for 10 minutes and permeabilized with methanol. Samples were blocked in 10% FCS in PBS for 1 hr and then were incubated overnight at 4°C with the primary antibody in blocking solution. After washes the coverslips were incubated with fluorescence labelled secondary antibody for 2 hours at room temperature, were mounted on slides, and examined by confocal microscopy. Immunofluorescence staining of Drosophila embryos and intact salivary glands after formaldehyde fixation, and methanol devitellinisation for embryos, was carried out using standard protocols; testes were stained as in [45]. For in situ hybridisation an anti-sense uri dig-labelled RNA probe was made by in vitro transcription using partial cDNA in pBluescript KS as a template, and Roche Dig-RNA labelling mix according to manufacturer’s instructions. The probe was hydrolysed to give an average length of 100 nucleotides, hybridisation was carried out as described [46].

**Staining of larval salivary gland polytene chromosomes**

Immunostaining of polytene chromosomes was performed as described by [47], with minor modifications [48]. For heat shock experiments, larvae were heat shocked at 37°C for 20 min in a water bath and salivary glands were dissected in PBS warmed to 37°C to prevent recovery. DNA was detected with Hoechst 33258 (0.5 µg/ml in water). Slides were mounted in 85% glycerol/PBS/2.5% n-propyl-gallate. Images were collected using a Q-imaging Retiga 1300 digital camera mounted on an Olympus BX50 epi-fluorescence microscope.

**RNA extraction and RT-PCR**

For reverse transcription PCR (RT-PCR) non-fluorescent uri\(^{110b}\) (or uri\(^{1}\)) homozygotes were sorted from their fluorescent uri\(^{110b}\) (or uri\(^{1}\))/CyO, act-GFP heterozygous siblings. For semi-quantitative RT-PCR on uri\(^{110b}\) three (18.5–20.5 hours after egg laying) embryos were pooled, and total RNA was extracted using Trizol (Invitrogen). cDNA was generated from all the extracted RNA using Superscript II (Invitrogen), and 1/40th of the cDNA reaction product was used as a template for each PCR (30 amplification cycles for ebony, 35 cycles for CG1315, CG3999, PP1/9C and GFP). To analyse expression of uri transcripts, and to map the 5’ end of uri, RNA was extracted from first instar larvae and processed as above.
TUNEL labelling of tests
TUNEL labelling was carried out with the "In situ cell death detection. Fluoroscein" kit (Roche). Tests were dissected from male larvae in testis buffer, transferred to a 20 µl drop of testis buffer on a poly-L-lysine treated slide, cut open, and 40 µl of 4% paraformaldehyde in PBS was added. After 15 minutes the cells were squashed by addition of a coverslip which was then flipped off after freezing in liquid nitrogen. Slides were washed briefly in PBS, permeabilised in PBS+ 1% Triton + 0.5% acetic acid, rinsed in PBS then permeabilised again in 0.1% Triton, 0.1% (Tri-)Na Citrate for 4 min. As a positive control one slide was treated with DNase for 10 minutes. Labelling was carried out for 1 hr at 37°C in a humid chamber using 5 µl enzyme solution and 45 µl label solution per slide. After washing with PBS the slides were incubated with 1 mg/ml RNase A for 15 min or 0.5 mg/ml RNase A overnight at 4°C, counter-stained with propidium iodide (1 µg/ml in PBS), mounted and observed with confocal microscopy.

Authors' contributions
JK made the uri mutant and did the phenotypic analysis. EV made the anti-Uri antibody and did immunostainings, immunoprecipitation and Western blotting. SG identified Uri as a PP1α specific binding protein. BS did the protein phosphatase assays. AR did immunostaining of polytene chromosomes. HW-C supervised aspects of the work, assisted with immunostainings, in situ hybridization and phenotypic analysis, and wrote the paper. LA cloned uri in the original yeast 2-hybrid screen did in situ hybridisation, and supervised aspects of the work. All authors read and approved the final version of the manuscript.

Acknowledgements
This work was funded by grants from the UK MRC and BBSRC to LA. HW-C is a Royal Society University Research Fellow.

We thank David Glover and Seishi Murakami for antibodies; Toshiro Aikagi for the P(GSV6)GS1/6344 fly stock; Dennis McKearin for the Bam-Gal4-PV16 line; Barry Dickson for RNAi lines; Seishi Murakami for the RMP and RMP-D2 clones; Steve Elledge for the yeast two-hybrid plasmid library; the Developmental Studies Hybridoma Bank (Iowa) for antibodies; Karen Clifton for technical assistance and members of the Zoology tests and phosphatase journal clubs for critical reading of the manuscript.

References
1. Raghavan S, Williams I, Aslam H, Thomas D, Szoor B, Morgan G, Gross S, Turner J, Fernandes J, Vijay-Raghavan K, Alphey L: Protein phosphatase 1beta is required for the maintenance of muscle attachments. *Curr Biol* 2000, 10:269-272.
2. Dombradi V, Axton JM, Barker HM, Cohen PT: Protein phosphatase 1 activity in Drosophila mutants with abnormalities in mitosis and chromosome condensation. *FEBS Lett* 1990, 275:39-43.
3. Vereshchagina N, Bennett D, Szoor B, Kirchner J, Gross S, Vissi E, White-Cooper H, Alphey L: The Essential Role of PP1β in Drosophila Is To Regulate Non-Muscle Myosin. * Mol Biol Cell 2004*, 15:4395-4405.
4. Clyne PJ, Certel SJ, de Bruyne M, Zaslavsky L, Johnson WA, Carlson JR: The odor specificities of a subset of olfactory receptor neurons are governed by AChβ1, a PDQ-domain transcription factor. *Neuron* 1999, 22:339-347.
5. Kirchner J, Gross S, Bennett D, Alphey L: Essential, overlapping and redundant roles of the Drosophila protein phosphatase 1 alpha and 1 beta genes. *Genetics* 2007, 176:273-281.
6. Ceulemans H, Bollen M: Functional diversity of protein phosphatase-1, a cellular economizer and reset button. *Physiol Rev* 2004, 84(1):1-39.
7. Munton R, Vizi S, Mansuy I: The role of protein phosphatase-1 in the modulation of synaptic and structural plasticity. *FEBS Lett* 2004, 567:121-128.
8. Nelson DA, Krucher NA, Ludlow JW: High molecular weight protein phosphatase type 1 dephosphorylates the retinoblastoma protein. *J Biol Chem* 1997, 272:4528-4535.
9. Li DW, Liu JP, Schmid PC, Schlosser R, Feng H, Liu WB, Yan Q, Gong L, Sun SM, Deng M, Liu Y: Protein serine/threonine phosphatase-1 dephosphorylates p53 at Ser-15 and Ser-37 to modulate its transcriptional and apoptotic activities. *Oncogene* 2006, 25:3006-3022.
10. Bollen M: Combinatorial control of protein phosphatase-1. *Trends Biochem Sci* 2006, 31:426-431.
11. Bennett D, Alphey L: PP1 binds Sara and negatively regulates Dpp signaling in Drosophila melanogaster. *Nat Genet* 2002, 31:419-423.
12. Bennett D, Lyulcheva E, Alphey L: Towards a comprehensive analysis of the protein phosphatase 1 interactome in Drosophila. *J Mol Biol* 2006, 364:196-212.
13. Cohen PTW: Protein phosphatase-1 targeted in many directions. *J Cell Sci* 2002, 115:241-256.
14. Andreassen PR, Lacroix FB, Villa-Moruzzi E, Margolis RL: Differential subcellular localization of protein phosphatase-1 alpha, gamma and delta isoforms during both interphase and mitosis in mammalian cells. *J Cell Biol* 1998, 141(1):1207-1215.
15. Lesage B, Beullens M, Ceulemans H, Himpens B, Bollen M: Determinants of the nuclear targeting of protein phosphatase-1. *FEBS Lett* 2005, 579:5626-5630.
16. Trinkle-Mulcahy L, Andrews PG, Wickramasinghe S, Sleeeman J, Prescott C, Lam YY, Lyon C, Swedlow JR, Lamond A: Time-lapse imaging reveals dynamic relocalization of PP1gamma within the mammalian cell cycle. *Mol Biol Cell* 2003, 14:107-117.
17. Trinkle-Mulcahy L, Sleeeman J, Lamond A: Dynamic targeting of protein phosphatase 1 within the nuclei of living mammalian cells. *J Cell Sci* 2001, 114:4219-4228.
18. MacMillan LB, Bass MA, Cheng N, Howard EF, Tamura M, Strack S, Wadzinski BE, Colbran RJ: Brain actin-associated protein phosphatase 1 holoenzymes containing spinophilin, neurabin, and selected catalytic subunit isoforms. *J Biol Chem* 1999, 274:35845-35854.
19. Terry-Lorenzo RT, Carmody LC, Volz JW, Connor JH, Li S, Smith FD, Milgram SL, Colbran RJ, Shenolikar S: The neuronal actin binding proteins, neurabin I and neurabin II, recruit specific isoforms of protein phosphatase-1 catalytic subunits. *J Biol Chem* 2002, 277:27716-27724.
20. Trinkle-Mulcahy L, Anderson J, Lam YY, Moorhead G, Mann M, Lamond A: Repo-Man recruits PP1 gamma to chromatin and is essential for cell viability. *J Cell Biol* 2006, 172:679-692.
21. Flores-Delgado G, Liu CW, Sposto R, Berndt N: A limited screen for protein interactions reveals new roles for protein phosphatase 1 in cell cycle control and apoptosis. *J Proteome Res* 2007, 6:1165-1175.
22. Gstaiger M, Luke B, Hess D, Oakeley EJ, Wirbelauer C, Blondel M, Vigneron M, Peter M, Krek W: Control of nutrient-sensitive transcription programmes by the unconventional prefoldin URI. *Science* 2003, 302:1208-1212.
23. Parusel CT, Kritikou EA, Hengartner MO, Krek W, Gotta M: URI-1 is required for DNA stability in C. elegans. *Development* 2006, 133:621-629.
24. Delgerma L, Hayashi N, Dorjsuren D, Nomura T, Thuy Le TT, Murakami S: Subcellular localization of RPB5-mediated protein and its putative functional partner. * Mol Cell Biol* 2004, 24:8556-8566.
acting protein, counteracts transactivation by hepatitis B virus X protein. Mol Cell Biol 1998, 18:7546-7555.

26. Van Leuven F, Torrekens S, Maechler D, Hilliker I, Buellens M, Bollen M, Delable J: Molecular cloning of a gene on chromosome 19q12 coding for a novel intracellular protein: analysis of expression in human and mouse tissues and in human tumor cells, particularly Reed-Sternberg cells in Hodgkin disease. Genomics 1998, 54:511-520.

27. Egloff MP, Johnson DF, Moorhead G, Cohen PT, Cohen P, Barford D: Structural basis for the recognition of regulatory subunits by the catalytic subunit of protein phosphatase 1. EMBO J 1997, 16:1876-1887.

28. Watanabe M, Buellens M, Cauletans H, Stalmans W, Bollen M: Degen
eracy and function of the ubiquitous RVXF motif that mediates binding to protein phosphatase-1. J Biol Chem 2003, 278:18817-18823.

29. Sanson B, White P, Vincent JP: Uncoupling cadherin-based ad hesion from wingless signalling in Drosophila. Nature 1996, 383:627-630.

30. Jamrich M, Greenleaf AL, Bautz EK: Localization of RNA polymerase in polytene chromosomes of Drosophila melanogaster. Proc Natl Acad Sci U S A 1977, 74:2079-2083.

31. Toba G, Ohsako T, Miyata N, Ohtsuka T, Seong KH, Aigaki T: The gene search system: A method for efficient detection and rapid molecular identification of genes in Drosophila melanogaster. Genetics 1999, 151:7-25-737.

32. Brand AH, Perrimon N: Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development 1993, 118:401-415.

33. Chen D, McKeearin DM: A discrete transcriptional silencer in the bam gene determines asymmetric division of the Drosophila germ line stem cell. Development 2003, 130:1159-1170.

34. Ni L, Snyder M: A genomic study of the bipolar bud site selection pattern in Saccharomyces cerevisiae. Mol Biol Cell 2001, 12:2147-2170.

35. Tronnerjsjo S, Hanelk C, Balciunas D, Hu GZ, Nordberg N, Murén E, Ronne H: The jmjN and jmjC domains of the yeast zinc fin
ger protein Gis1 interact with 19 proteins involved in transcrip
tion, sumoylation and DNA repair. Mol Genet Genomics 2007, 277(1):57-70.

36. Zhang H, Stalllock JP, Ng JC, Reinhard C, Neufeld TP: Regulation of cell growth by the Drosophila target of rapamycin dTOR. Genes Dev 2000, 14:2712-2724.

37. Bauer A, Chauvet S, Huber O, Usseglio F, Rothbacher U, Aragoni D, Kemler R, Pradel J: Pontin52 and Reptin52 function as antago
nist regulators of β-catenin signalling activity. EMBO J 2000, 19:6121-6130.

38. Giot L, Bader JS, Brouwer C, Chaudhuri A, Kuang B, Li Y, Hao YL, Zhu Z, Martin D, Bader JS, Brouwer C, Chaudhuri A, Kuang B, Li Y, Hao YL, Zhu Z, Martin D, Van Mieghem C, Bolle M, Guettouche T, Willems V, Suprangan P, Tyers M, Rickert S, Donaldson I, Sharan R, Wheeler R, Sander C, Topological and functional characterization of chicken protein-protein interaction networks. Proceedings of the National Academy of Sciences 2003, 100:15579-15584.

39. Grumblin G, Strelets V, Consortium TFB: FlyBase: anatomica data, images and queries. Nucleic Acids Research 2006, 34:D484-D488.

40. Harper JW, Adami GR, Wei N, Keyomarsi K, Elledge SJ: The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cycl

41. Alphey L, Park L, Hawcroft G, Guo Y, Kaiser K, Morgan G: KLPI38B: a mitotic kinesin-related protein that binds PPI. J Cell Biol 1997, 138:495-499.

42. Parker L, Gross S, Alphey L: Vectors for the expression of tagged proteins in Drosophila. Biotechniques 2001, 31:1280-2, 1284, 1286.

43. Berndt N, Cohen PT: Retinaturation of protein phosphatase 1 expressed at high levels in insect cells using a baculovirus vector. Eur J Biochem 1990, 190:291-297.

44. Szoor B, Gross S, Alphey L: Biochemical Characterization of Recombinant Drosophila Type I Serine/Threonine Protein Phosphatase (PPI) Produced in Pichia pastoris. Arch Biochem Biophys 2001, 396:213-218.

45. Jiang J, White-Cooper H: Transcriptional activation in Dro-

46. White-Cooper H: Spermatogenesis: analysis of meiosis and morphogenesis. In Drosophila Cytogenetics Protocols Volume 247. Edited by: Henderson D, Totowa, New Jersey, Humana Press; 2004:45-75.

47. Armstrong JA, Papoulas O, Daubregrasse S, Sperling AS, Lis JR, Scott MP, Tamkun JW: The Drosophila BRM complex facilitates global transcription by RNA polymerase II. EMBO J 2002, 21:5245-5254.

48. Rudenko A, Bennet D, Alphey L: Trithorax interacts with the T

Publish with BioMed Central and every scientist can read your work free of charge

"BioMed Central will be the most significant development for disseminating the results of biomedical research in our lifetime. Sir Paul Nurse, Cancer Research UK

Your research papers will be:

- available free of charge to the entire biomedical community
- peer reviewed and published immediately upon acceptance
- cited in PubMed and archived on PubMed Central
- yours — you keep the copyright

Submit your manuscript here:
http://www.biomedcentral.com/info/publishing_adv.asp