Supplemental Information

Sequential Regulation of Maternal mRNAs through a Conserved cis-Acting Element in Their 3’ UTRs

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Supplemental Figure 1. Pgc is translationally regulated via its UTRs (Related to Figures 1 and 2) (A) The ovariole of a pgc mutant fly probed for pgc RNA (magenta) using FISH, show no signal for pgc RNA. (B) The ovariole of a pgcGFP transgenic fly probed for GFP RNA (magenta) using FISH, show similar expression pattern when compared to endogenous pgc RNA. (C) The ovariole of a wild-type fly probed for GFP RNA (magenta) using FISH, show no signal for GFP RNA. (D) RT-PCR of pgc CDS was carried out on RNA samples extracted from wild-type, nosGAL4>UAStkv and nosGAL4>bamRNAi show pgc RNA is not only present in whole adult ovaries, but also transcribed in GSC and CB enriched tumors. RNA null pgc mutant was used as a negative control. RT-PCR of Vasa was carried out as a positive control. (E) RT-PCR of pgc 5′UTR was carried out on RNA samples extracted from wild-type, nosGAL4>UAStkv and nosGAL4>bamRNAi. Primers were designed as to show either a 788bp or a 263bp product to confirm what 5′UTR length of pgc RNA was being expressed during oogenesis. Results showed presence of short version of pgc 5′ UTR in whole adult ovaries, GSC and CB enriched tumors. RNA null pgc mutant was used as a negative control. RT-PCR of Vasa was carried out as a positive control. (F) The ovariole of a transgenic fly created by fusing GFP to the pgc 5′ and tub 3′UTR and under the control of the pgc promoter was stained with 1B1 (red) which marks the spectrosomes and fusomes, Vasa (blue) which marks the germline and GFP (green) which marks Pgc expressing cells. There is a loss of GFP regulation throughout oogenesis, including at the earliest stages (yellow dashed line). (G) A phylogenetic analysis of pgc 3′UTR of all Drosophilids identified a conserved sequence that can potentially bind both RBPs, Pum and Bru. (H) The ovariole of a transgenic fly created by fusing GFP to pgc 5′ and pgc 3′UTR where the UGUA sequence was mutated to UCUC (3′UTR: UCUCAAUU) and driven under pgc promoter stained with 1B1 (red), Vasa (blue) and GFP (green) shows loss of GFP regulation throughout oogenesis. GFP channel shown in H1. (I) Ovariole of a transgenic fly created by fusing GFP to pgc 5′ and pgc 3′UTR where the UGUA sequence was deleted (3′UTR: AUGUA) and driven under pgc promoter stained with 1B1 (red), Vasa (blue) and GFP (green) shows loss of GFP regulation throughout oogenesis. GFP channel shown in I1. (J) Normalized protein expression to RNA levels shows that either deletions or mutations in the PRE/BRE sequence of the 3′UTR of pgc results in a significant upregulation of Pgc reporter protein when compared to FL 3′UTR. The graph represents an average generated from three independent biological replicates. The error bars are the standard error calculated from these replicates. A student t-test statistical analysis was performed. * indicates p-value <0.05 and ** indicates p-value <0.005. Scale bars: 10μm.
Flora_Figure S2

A) pgc promoter 5'UTR eGFP tub 3'UTR

B) pgc promoter 5'UTR eGFP tub 3'UTR: NBS+PRE/BRE

C) Bar graph showing protein level relative to RNA level for tub 3'UTR and tub 3'UTR: NBS+PRE/BRE.

D) Coomassie of purified proteins

E) Western Blot of RIPs

F) GFP Bru 1B1

F1) GFP Bru 1B1

F2) GFP Bru 1B1

F1') GFP Bru 1B1

F2') GFP Bru 1B1

GFP Bru 1B1

Bru
Supplemental Figure 2. A cis-element in the pgc 3’UTR that binds both Pum and Bru is required for translational control throughout oogenesis (Related to Figure 2) (A) The ovariole of a transgenic fly created by fusing GFP to the pgc 5’ and tub 3’UTR and under the control of the pgc promoter stained with 1B1 (red) which marks the spectrosomes and fusomes, Vasa (blue) which marks the germline and GFP (green) shows a loss of GFP regulation throughout oogenesis (yellow dashed line). GFP channel is shown in A1. (B) The ovariole of a transgenic fly created by fusing GFP to the pgc 5’ and tub 3’UTR that contains the NBS and PRE sequences and under the control of the pgc promoter stained with 1B1 (red), Vasa (blue) and GFP (green). Insertion of the sequence is sufficient translation repression. (C) Normalized protein expression to RNA levels shows that insertion of the NBS+PRE/BRE sequence in the tub 3’UTR results in significant repression of reporter protein when compared to control. The graph represents an average generated from three independent biological replicates. The error bars are the standard error calculated from these replicates. A student t-test statistical analysis was performed. * indicates p-value <0.05. (D) Commasie stained SDS-PAGE gels shows successful purification of recombinant Pum (left) and recombinant Bru protein (right). (E) Western Blot shows successful pull-down of Pum (top) and Bru (bottom) from wild-type ovary lysates using anti-Pum and anti-Bru antibody, respectively. (F-F2’) pumGFP transgene fly stained with Bru (red), 1B1 (blue) and GFP (green) which marks Pum expressing cells shows that Pum protein is expressed in high levels in the earliest stages of oogenesis and lowers in later differentiating stages while Bru protein levels are low in early stages and increases from the 8-cell cyst stages and onwards. F1-F1’ and F2-F2’ shows GFP and Bru channels in gray. Scale bars: 10μm.
Supplemental Figure 3. Pum and its co-factor Nos regulate Pgc translation in the GSCs (Related to Figure 3)

(A, A1) The gerarium of pgcGFP fly stained with Pum (red), 1B1 (blue) which marks fusomes and spectrosomes and GFP (green) which marks Pgc expressing cells shows high levels of Pum protein in the GSC, pre-CB (yellow dashed circle) 2- to 4-cell cysts. Pum staining is shown in gray in A1. (B, B1) The gerarium of pgcGFP fly stained with Nos (red), 1B1 (blue) and GFP (green) which marks Pgc expressing cells shows Nos protein is present throughout the gerarium except for the GFP expressing pre-CB cell (yellow dashed circle). Nos staining is shown in gray in B1. (C) The gerarium of pgcGFP fly stained with pMad (red) which marks GSCs, 1B1 (blue) and GFP (green) shows GSCs do not express GFP (yellow arrow). (D-F) The geraria of pgcGFP, pgcGFP; pum, pgcGFP; nos and pgcGFP; twin stained with pMad (red), 1B1 (blue) and GFP (green) show that in absence of Pum and its co-factors, there is a loss of GFP regulation in the GSCs (yellow arrow). (G-I) The geraria of pgcGFP; nosGAL4>pumRNAi, pgcGFP; nosGAL4>nosRNAi and pgcGFP; nosGAL4>twinRNAi flies stained with 1B1 (red), Vasa (blue) and GFP (green) show aberrant expression of GFP in the earliest stages of oogenesis, including the GSCs (outlines in yellow dashed line). (J, J1) The gerarium of nosGAL4 flies stained with 1B1 (red), Vasa (blue) and Pum (green) shows Pum being expressed in high levels in somatic cells and in the earliest stages of oogenesis. Pum channel shown in J1. (K, K1) The gerarium of nosGAL4; pumRNAi flies stained with 1B1 (red), Vasa (blue) and Pum (green) shows Pum is significantly downregulated in the ovaries that contain germline. Pum channel shown in K1. (L, L1) The gerarium of germline depleted not1 ovary stained with 1B1 (red), Vasa (blue) and GFP (green) show aberrant expression of GFP in the GSCs and 4-cell cysts (100%, n = 25 germaria) (outlined in yellow dashed line). GFP channel showed in gray scale in A1. (M, M1) The gerarium of germline depleted pop2 ovary stained with 1B1 (red), Vasa (blue) and GFP (green) shows aberrant expression of GFP in the GSCs to the 4-cell cyst stages (100%, n = 25 germaria) (outlined in yellow dashed line). GFP channel showed in gray scale in B1. (N) A western blot analysis shows a significant upregulation of Pgc reporter protein in the germline depletion of pum, nos, twin, not1, and pop2 ovaries when compared to pgcGFP. The graph represents an average generated from three independent biological replicates. The error bars are the standard error calculated from these replicates. A student t-test statistical analysis was performed. * indicates p-value <0.05 and ** indicates p-value <0.005. (O, O1) The gerarium of nosGAL4.NGT>UAS-GFP stained with 1B1 (red), Vasa (blue) and GFP (green) shows no difference in GFP expression levels in the gerarium. GFP channels shown in O1. (P, P1) The ovariole of nosGAL4.VP16>UAS-GFP stained with 1B1 (red), Vasa (blue) and GFP (green) shows no difference in GFP expression levels in the gerarium. GFP channels shown in P1. Scale bars: 10μm.
Flora_Figure S4

A. $\text{nosGAL4}\neg\text{UAStkv}$; B. $\text{nosGAL4}\neg\text{UAStkv};\ pum\neg\text{RNAi}$; C. $\text{nosGAL4}\neg\text{UAStkv};\ \text{pgcHA}$.

D. $\text{me31BGFP}\neg\text{nosGAL4}$; E. $\text{me31BGFP}\neg\text{me31BRNAi}$.

F. Relative HA levels.

** corresponds to a significant difference.
Supplemental Figure 4. Me31B cooperates with the decapping protein dGe-1 and pge 5'UTR to mediate repression in the GSCs and early differentiating cysts (Related to Figures 3 and 4) (A, A1) The germarium of nosGAL4>UAS-tkv ovary stained with 1B1 (red) which marks the spectrosomes and fusomes, Vasa (blue) which marks the germline and pMad (green) which marks GSCs shows a tumor enriched with GSCs. pMad channel shown in A1. (B-C1) The germaria of nosGAL4>UAS-tkv; pumRNAi, nosGAL4>UAS-tkv; nosRNAi ovary stained with 1B1 (red), Vasa (blue) and pMad (green) shows a tumor of enriched with GSCs. pMad channel shown in B1 and C1. (D, D1) The germarium of me31BGFP-trap; nosGAL4 ovary stained with 1B1 (red), Vasa (blue) and GFP (green) which marks Me31B expressing cells shows Me31B being expressed in both the germline and somatic cells of the germarium. GFP channel shown in D1. (E, E1) The germarium of me31BGFP-trap; nosGAL4 depleted of me31B via RNAi stained with 1B1 (red), Vasa (blue GFP (green) Me31B being expressed only in the somatic cells of the germarium confirming germline knockdown of Me31B via RNAi. GFP channel shown in E1. (F) A western blot analysis shows a significant upregulation of Pgc reporter protein in the germline depletion of dGe-1 ovaries when compared to pgcGFP. The graph represents an average generated from three independent biological replicates. The error bars are the standard error calculated from these replicates. A student t-test statistical analysis was performed. * indicates p-value <0.05 and ** indicates p-value <0.005. We were unsuccessful in isolating stable lysates from Me31B depleted ovaries to carry out a WB analysis. Scale bars: 10μm.
**Flora Figure S5**

### A

**Relative HA Levels**

| Condition         | Relative HA Level |
|-------------------|------------------|
| WT                | 1.0              |
| pgcHA             | 0.6              |
| pum<sup>680</sup> | 0.6              |
| nosGAL4>>d4EHPRNAi| 1.4              |
| nosGAL4>>bratRNAi | 1.0              |

### B

**Product Length and Intensity (A.U.)**

- **pgc**
  - WT: 400, 700, 1000 A.U.
  - pum<sup>680</sup>: 400, 700, 1000 A.U.

- **actin**
  - WT: 400, 700, 1000 A.U.
  - nosGAL4>d4EHPRNAi: 400, 700, 1000 A.U.
  - nosGAL4>>bratRNAi: 400, 700, 1000 A.U.
Supplemental Figure 5. Pum and its co-factor Brat regulate Pgc translation in the 4- to 16-cell cysts (Related to Figure 5) (A) A western blot analysis shows a significant upregulation of Pgc reporter protein in pum680 and the germline depletion of brat and d4EHP ovaries when compared to pgcGFP. The graph represents an average generated from three independent biological replicates. The error bars are the standard error calculated from these replicates. A student t-test statistical analysis was performed. ** indicates p-value <0.005. (B) PAT assay analysis of pgc poly(A)-tail length in wild-type, pum680 and germline depletions of d4EHP and Brat show that loss of these factors do not result in any change of poly(A)-tail length of pgc.
Supplemental Figure 6. Bru and Cup regulate Pgc translation in the later stages of oogenesis (Related to Figure 6) (A, A1) The ovariole of *pgcGFP; bruRNAi*QB/PA stained with 1B1 (red) which marks the spectrosomes and fusomes, Vasa (blue) which marks the germline and GFP (green) which marks Pgc expressing cells shows upregulation of reporter expression from 16-cell cyst onwards (outlined in yellow dashed line). GFP channel shown in A1. (B) A western blot analysis shows a significant upregulation of Pgc reporter protein in the germline depletion of Bru ovaries when compared to *pgcGFP*. We were unsuccessful in isolating stable lysates from Cup depleted ovaries to carry out a WB analysis. The graph represents an average generated from three independent biological replicates. The error bars are the standard error calculated from these replicates. A student t-test statistical analysis was performed. ** indicates p-value <0.005. (C, C1) The ovariole of control *nosGAL4* ovary stained with 1B1 (red), Vasa (blue) and Bru (green) shows Bru being expressed from 16-cell cyst and onwards. Bru channel shown in C1. (D, D1) The ovariole of *nosGAL4>bruRNAi* stained with 1B1 (red), Vasa (blue) and Bru (green) shows little or no Bru expression in the ovariole. GFP channel shown in D1. Scale bars: 10μm.
Flora_Figure S7

A

Young nosGAL4
Cyclohexamide treatment

pumRNAi
Cyclohexamide treatment

bruRNAi
Cyclohexamide treatment

B

B

C

C

pgcGFP

pgcGFP;pum & pumRNAi

pgcGFP;nos & nosRNAi

pgcGFP;twin & twinRNAi

pgcGFP;me31bRNAi

pgcGFP;dGe-1RNAi

pgcGFP;pum<sup>neo</sup>

pgcGFP;bratRNAi

pgcGFP;d4EHPRNAi

pgcGFP;bruRNAi

pgcGFP;cupRNAi

Region of overlap

Bits

0 1 2

1 2 3 4 5 6 7 8 9 10

No GFP expression

<50% GFP expression

>50% GFP expression

Wild-type expression

Germ line death

Stage 1-9

GSC

CB

2-cell Cyst

4-cell Cyst

8-cell Cyst

16-cell Cyst

Region of overlap

pumRNAi

bruRNAi

nosGAL4
Supplemental Figure 7. A class of germline RNAs are similarly regulated by both Pum and Bru (Related to Figure 7) (A) Polysome profile traces of young wild-type, pgeGFP; nosGAL4>pumRNAi, and pgeGFP; nosGAL4>bruRNAi ovaries treated with cyclohexamide. (B) The logo of the sequences used to identify shared targets of Pum and Bru mediated regulation that contain a sequence similar to the PRE/BRE sequence identified in the pge 3’UTR. (C) A developmental profile of GFP expression in pgeGFP, pgeGFP; pumET1/FCN and germline knockdown of pum, pgeGFP; nosRC/BN and germline knockdown of nos and pgeGFP; twin3/ry5 and germline knockdown of twin, pgeGFP; me31BRNAi, pgeGFP; dGe-IRNAi, pgeGFP; pum680, pgeGFP; nosGAL4>brarRNAi, pgeGFP; nosGAL4>d4EHPRNAi, pgeGFP; nosGAL4>bruRNAi, and pgeGFP; nosGAL4>cupRNAi ovarioles show temporal and sequential loss of GFP regulation in different stages of oogenesis where these trans-acting factors mediate pge regulation.