**hsp-90 and unc-45 depletion induce characteristic transcriptional signatures in coexpression cliques of C. elegans**

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Nematode development is characterized by progression through several larval stages. Thousands of genes were found in large scale RNAi-experiments to block this development at certain steps, two of which target the molecular chaperone HSP-90 and its cofactor UNC-45. Aiming to define the cause of arrest, we here investigate the status of nematodes after treatment with RNAi against *hsp*-90 and *unc*-45 by employing an in-depth transcriptional analysis of the arrested larvae. To identify misregulated transcriptional units, we calculate and validate genome-wide coexpression cliques covering the entire nematode genome. We define 307 coexpression cliques and more than half of these can be related to organismal functions by GO-term enrichment, phenotype enrichment or tissue enrichment analysis. Importantly, *hsp*-90 and *unc*-45 RNAi induce or repress many of these cliques in a coordinated manner, and then several specifically regulated cliques are observed. To map the developmental state of the arrested nematodes we define the expression behaviour of each of the cliques during development from embryo to adult nematode. *hsp*-90 RNAi can be seen to arrest development close to the L4 larval stage with further deviations in *daf*-16 regulated genes. *unc*-45 RNAi instead leads to arrested development at young adult stage prior to the programmatic downregulation of sperm-cell specific genes. In both cases processes can be defined to be misregulated upon depletion of the respective chaperone. With most of the defined gene cliques showing concerted behaviour at some stage of development from embryo to late adult, the "clique map" together with the clique-specific GO-terms, tissue and phenotype assignments will be a valuable tool in understanding concerted responses on the genome-wide level in *Caenorhabditis elegans*.

Nematode-development is a highly complex process that is defined by temporal and spatial events in different tissues and cell types. Therefore simultaneous events are occurring in this process with chronological timing to enable the highly reproducible development program.

HSP-90 (DAF-21) is a molecular chaperone, crucial for the development of vulva, gonads and oocyte maturation as well as ensuring longevity of *C. elegans*\(^1\)\(^-\)\(^3\). It is an indispensable protein, activating and regulating many clients, for example protein kinases, and transcription factors, such as steroid receptors\(^4\)\(^-\)\(^6\). Inhibition of HSP-90, by either RNAi or specific compounds, therefore has the potential to interfere with several pathways. RNAi arrests the nematode development and reduces motility in later larval stages\(^7\)\(^,\)\(^8\). Prominent responses induced after *hsp*-90/*daf*-21 RNAi include the heat-shock response, which is known to be suppressed by HSP-90 in most organisms\(^1\)\(^,\)\(^9\)\(^,\)\(^10\). Other affected responses are potentially regulated in a more organism-specific manner, like the innate immune response, which is coupled to the heat-shock response in nematodes\(^11\)\(^,\)\(^12\). Interestingly, both of these responses are also dependent on the developmental state of the nematode, with the heat-shock response being barely inducible in early larvae and also in adult aging nematodes\(^11\). The reason for these correlations is unclear, but it could be supported by assigning genes clearly to individual responses, so that the common principles and regulatory patterns become obvious.

The HSP-90 cofactor UNC-45 participates in the muscle-specific functions of HSP-90. Invertebrates possess a single *unc*-45 gene, which is expressed in muscle cells, where UNC-45 performs HSP-90-dependent folding of the myosin motor domain. It further is expressed in non-muscle tissues of early embryos\(^13\)\(^-\)\(^15\). Depletion of the...
HSP-90-cofactor UNC-45 leads to rather specific morphological changes, like paralysis due to muscle cell defects and sterility in C. elegans7. To see, whether these interacting factors influence common pathways, we compare the transcriptional response to depletion of these two proteins by microarray analysis. Microarrays are high-throughput analyses yielding a snapshot of the expression status of each represented gene16. For C. elegans a wealth of data exists, which link different sample conditions to the induction of certain marker genes. Here, as performed before for yeast17, we derive and validate genome-wide coexpression cliques and use statistical analyses to define the cliques responding to hsp-90 and unc-45 RNAi treatment.

Material and methods

Constructing genome-wide coexpression networks for yeast has been described in detail before17. This genome-wide nematode gene network was then used to extract the individual cliques by isolating high density areas in an automated fashion from the network as described before17. Altogether 307 cliques were obtained, with the largest clique containing 1327 genes and the smallest clique containing 5 genes. The nematode analysis methods are added to the webserver functionality.

GO-term, phenotype and tissue enrichment. GO-term enrichment was analysed to test, whether some of the 307 aforementioned cliques enrich genes with functional similarity. To this end also published information from phenotype and tissue enrichment studies was used. As such the associations between genes and GO-terms were obtained from the “go_dictionary.csv” table available from https://github.com/dangelles/TissueEnrichmentAnalysis21. For phenotype enrichment the table “phenotype_ontology.csv” was employed (PEA22) and for tissue enrichment the tissue sets designated as “genesets_golden” were utilized. In all cases the calculation of the enrichment was performed as described21 (Supplemental Table 2). 20 randomly scrambled clique sets were generated to determine, whether enrichment is considered relevant up to p-values of 1e-3, 1e-4 or beyond 1e-5.

Gene-expression changes after RNAi against hsp-90 and unc-45. RNAi was used to deplete nematodes of hsp-90 or unc-45 mRNA and to induce the growth arrest and the transcriptomic responses of the nematodes. RNAi-treated nematodes were washed off the plates and were shock frozen immediately. Microarray experiments were performed at the Zentrum für Fluoreszenten Proteinanalytik in Regensburg. To study the response to hsp-90 RNAi or unc-45 RNAi we analysed independent biological replicates. In these experiments, RNAi did not always yield the same level of growth arrest in the case of hsp-90, where the first microarray experiment produced a weaker response. We used each experiment sample/control-pair to assign all its differential expression values to the coexpression cliques and analysed those in respect to significant induction or repression. As the RNAi experiments were analysed on the more rarely employed GPL19290 Affymetrix platform (Affymetrix C. elegans Gene 1.0 ST Array), we bridged the cliques obtained from GPL200 ProbeSets to the GPL19230 ProbeSets. This bridging was performed by employing the given gene names without the ProbeSet-specific indexing. If a gene was represented by more than one ProbeSet in the cliques, then each of those instances was given the value determined from the GPL19230 expression data. If on the other hand, only one GPL200-derived ProbeSet was present in the cliques and several ProbeSets for this gene are recoded on the GPL19230 arrays, then the GPL19230-values were averaged and this value was used to color the clique map and to derive the statistical parameters for the clique. If the same gene contained two different probes on both platforms, then the averaged GPL19230-value was used in both occurrences in the clique map. 1603 ProbeSets of the “clique map” did not receive data from GPL19230 this way and had to be omitted in the analysis. Despite these bridging needs between the platforms, significant changes in many cliques can be detected in each analysed RNAi experiment. The observed experiments were also analysed with the Transcriptome Analysis Console (TAC, Thermo Fisher Scientific) as a state-of-art method for analysis of microarray data.

Statistical analysis employing the clique map was done as described before21. In short, color coding of the clique set figures was done by determining the differential values for each gene and then assigning discrete values between −4 and +4 for the transcriptional changes of log2 < −1 to log2 > +1. For each discrete value a red tone
or blue tone was defined in Cytoscape (https://cytoscape.org/)\(^2\). In cases where responses were weak, like both unc-45 experiments, the scale was adjusted to reach from log2 < −0.25 to log2 > +0.25. This analysis leads to information on most cliques as to whether they are induced or repressed with statistical significance as described before\(^7\). This method to evaluate nematode array data will be implemented for public use in the clusterEX.de webserver, which currently has this functionality only for yeast arrays.

Correlation analysis between different samples was made by plotting the cliques’ expression values against each other and obtaining the coefficient of determination \(R^2\) for the regression line. If \(R^2\) was closer to 1, the correlation between the two sets was considered to be better. These results were compared to correlations on the gene level in cases where identical array types were utilized.

**Analysis of microarray data on development.** To define moments of clique relevance during development, time points from developmental series were used to determine a transcriptional status for each clique in the map. In many cases, cliques react to developmental steps as concerted units resulting in a non-random distribution of up- and downregulated hits throughout the 307 cliques. To cover several larval states, three published GPL200 series were obtained from the GEO repository. These represent a time course of early development with data from embryo, L1 and L4 larvae (GSE654724) and a time course describing the aging process with time points at L4 larvae, and adults at day6 and day15 of development (GSE217844\(^2\)). Lastly, a time-course was included describing different stages of larval development, composed of L3, L3-lethargus, L4, L4-lethargus and young adult (GSE462914\(^2\)). Expression values were obtained from the normalized data table containing all public GPL200 experiments (see above).

**Results**

A genome-wide coexpression clique map for the nematode *C. elegans.* To obtain transcriptional units influenced by *hsp-90* and *unc-45* RNAi-treated nematodes, we first generated gene cliques that are coregulated in *C. elegans*, in which each of the 22,620 genes is assigned to exactly one clique. We had used the same procedure before to generate a coexpression clique map for *S. cerevisiae\(^7\). Based on the same stepwise procedure, we grouped every gene reported on standard microarrays of the GPL200 platform into one coexpression clique of at least five genes. The procedure resulted in 307 coexpression cliques, which were visualized in Cytoscape to generate the “coexpression clique map” for *C. elegans*. We set out to test, whether these 307 coexpression cliques are gene groups with a high level of functional similarity, as it was observed for the yeast clique map before\(^7\).

Therefore, we investigated all cliques by GO-term enrichment analysis. 220 of the 307 cliques show a GO-term enrichment with a \(p\)-value lower than 1e–4, 172 showed less than 1e–4, and 148 of the 307 cliques showed \(p\)-values of less than 1e–5 (Best results in Table 1). This is far better than 307 random cliques, which yielded these \(p\)-values 18 times, 2 times and zero times. We also found significant enrichments employing phenotype enrichment analysis (PEA\(^2\)) and tissue enrichment analysis (TEA\(^2\)) with 145, 106 and 81 cliques being enriched for the same phenotype (20, 3 and 0 times in random cliques) and 45, 37 or 29 cliques being enriched for tissue-specific expression in the three \(p\)-value categories (0, 0, and 0 times in random cliques). The values also are far better than cliques composed of random genes. In this way, roughly two thirds of the 307 coexpression cliques were assigned either a function, a related phenotype or a tissue-specific expression with acceptable significance criteria of below 1e–4 (Table 1).

*hsp-90* RNAi affects embryo development and induces stress responses. Having confirmed that the “clique map” of coexpressed genes also holds information on functional, phenotypic and tissue-specific signatures, we set out to investigating the transcriptional response of *hsp-90* RNAi-treated nematodes. We previously had analysed these microarray data based on the Top250 differential regulated genes obtained from three experiments\(^1\). *hsp-90* depleted nematodes showed sterility, incomplete development of gonad arms and the formation of endomitotic oocytes\(^7\). Development is mostly blocked at a later larval stage. TAC analysis revealed many genes with substantially different expression levels and showed the strongest response in the experiment 1 (P152), while the experiment 2 (A966) and 3 (P062) showed a weaker response (Fig. 1a). Gene expression changes had implied the induction of the heat-shock response and the innate immune response in analyses before\(^1\), but due to the focus on only 500 of the 22,620 genes measured with this array type, information from the many weaker affected genes could not be considered in this study\(^1\).

The genome-wide gene expression cliques as defined here, instead allow visualization and analysis of all values. Significance analysis showed enrichment of up- and downregulation in many cliques for experiment 1 (Fig. 1b), but also for the other experiments 2 and 3 (Supplemental Fig. 1b and c). Indeed almost half of the cliques respond to the RNAi-treatment with a concerted response of their genes (Best cliques in Table 2). We first determined the upregulated cliques: these contain the clique col-138-col-49, which is holding genes related to the “structural constituent of cuticle” and the clique abu-7-abu-8_22491 related to the “response to unfolded protein”. The largest upregulated clique, containing 209 genes, is mlt-9_22518-F33D4.6_14044 (‘cuticle development” and phenotype of “molt variant” and localized in the “embryo hypodermis”) and other cliques related to cuticle formation, including R12E2.14_75-R12E2.15, col-117-col-167_1015, col-146-col-133, col-128-chd-10_9234 (enriched in “peptidase activity”) and sqt-2-dpy-9. Cliques which are strongly upregulated also in experiment 2, include the cliques related to the “immune system response” C10C5.2-Y58A7A.3 and K08D10.9-F46A8.1. Based on the assignment of GO-terms, phenotypes and tissues, the largest and strongest downregulated cliques (Table 2) represent “embryo development” (T22D1.5-inx-14, enriched phenotype of “aneuploidy” and localized in “embryonic germline precursors”), “embryo development” (T24D1.3-egg-1, enriched phenotype of “polynomial body defective early embryo”) and “reproduction” (puf-3-oma-2_18268, enriched phenotype “meiotic chromosome segregation variant” localized in the “germline_precursors”) among many other cliques hinting at the stalled
| Cluster number | Cluster name | Clinic position | Best GO-term | log10(pvalue), GO | Enrichment-Fold_GO | Best PEA-Term | log10(pvalue), PEA | Enrichment-Fold_PEA | Best TEA-Term | log10(pvalue), TEA | Enrichment-Fold_TEA |
|----------------|--------------|-----------------|--------------|-------------------|-------------------|---------------|-------------------|-------------------|---------------|-------------------|-------------------|
| 80             | rps-14_21270-rps-11_20714 | R4 C17        | Structural constituent of ribosome GO:0003735 | 189.66            | 83.29             | Plosetropic defects severe early emb WPPheno-type:0.001270 | 120.69          | 43.09           | WBPaper0026900, intestine enriched, WBBt:0005772_1970 | 1.44           | 1.28           |
| 211            | slr-42-owr-113 | R1 C1          | Sensory perception GO:0007400 | 143.77            | 6.31              | Dauer metabolite variant WPPheno-type:0.001547 | 10.86           | 2.84            | WBPaper0040420, FLP enriched, WBBt:0006828_298 | 0.00           | 0.46           |
| 282            | slr-21-qvb-32 | R1 C3          | Intrinsic component of membrane GO:0031214 | 128.75            | 2.29              | Dauer metabolite variant WPPheno-type:0.001547 | 7.14            | 3.04            | WBPaper0040420, FLP enriched, WBBt:0006828_298 | 0.00           | 0.37           |
| 187            | F43H10.2-R53.4.21676 | R3 C4         | Organelle inner membrane GO:0019866 | 74.24             | 34.23             | Avoids bacterial lawn WPPheno-type:0.000402 | 27.00           | 6.57            | WBPaper0037950, all-neurons larva enriched, WBBt:0003679_1013 | 3.37           | 1.50           |
| 93             | fdp-5-F17C11.2 | R4 C4          | Neuropeptide signaling pathway GO:0007218 | 56.65             | 67.09             | Sinusoidal movement variant WPPheno-type:0.000402 | 22.79           | 11.85           | WBPaper0037950, all-neurons larva enriched, WBBt:0003679_1013 | 22.28          | 3.93           |
| 90             | slr-1-T09B9.3 | R1 C5          | Intrinsic component of membrane GO:0031224 | 52.94             | 1.97              | Sinusoidal movement variant WPPheno-type:0.000402 | 8.60            | 3.85            | WBPaper0037950, all-neurons larva enriched, WBBt:0003679_1013 | 53.78          | 4.32           |
| 42             | rab-28-pta-14_9542 | R3 C5         | Cell projection assembly GO:0030031 | 45.54             | 56.43             | Amphipod phasmid sensorium morphologype variant WPPheno-type:0.001527 | 16.40           | 17.48           | WBPaper0037950, BAG-neuron,embryo_enriched, WBBt:0008255_454 | 42.10          | 7.48           |
| 203            | npr-33-ZK1025.1.8337 | R1 C2         | Sensory perception GO:0007600 | 45.42             | 4.04              | Dauer metabolite variant WPPheno-type:0.001547 | 6.13            | 2.24            | WBPaper0037950, coelomocytes larva enriched, WBBt:0005751_229 | 0.02           | 0.64           |
| 84             | cod-84-col-45 | R9 C17         | Collagen trimmer GO:0005270 | 44.38             | 77.74             | Dumpy WPPheno-type:0.000583 | 3.04            | 8.11            | WBPaper0040420, FLP enriched, WBBt:0006828_298 | 0.00           | 0.20           |
| 41             | cos-177-cos-167.1015 | R9 C25        | Structural constituent of cuticle GO:0042302 | 39.93             | 83.10             | Dumpy WPPheno-type:0.000383 | 5.47            | 7.68            | WBPaper0057950, germline-precursors, embryo_enriched, WBBt:0006849_974 | 0.00           | 0.33           |
| 233            | his-46_859-his-64 | R10 C26       | DNA packaging complex GO:0044815 | 39.57             | 191.32            | Sister chromatid segregation defective early emb WPPheno-type:0.000772 | 26.48           | 94.19           | WBPaper0037950, germline-precursors, embryo_enriched, WBBt:0006849_974 | 0.00           | 0.07           |
| 215            | cod-138-col-49 | R9 C23         | Structural constituent of cuticle GO:0042302 | 35.30             | 87.06             | Blistered WPPheno-type:0.000625 | 5.77            | 41.59           | WBPaper0037950, coelomocytes larva enriched, WBBt:0005751_229 | 0.07           | 0.60           |
| 80             | rps-14_21270-rps-11_20714 | R4 C17        | Structural constituent of ribosome GO:0003735 | 189.66            | 83.29             | Plosetropic defects severe early emb WPPheno-type:0.000270 | 120.69          | 43.09           | WBPaper0026900, intestine enriched, WBBt:0005772_1970 | 1.44           | 1.28           |
| 31             | pbs-3_18439-rps-5 | R3 C7          | Modification-dependent macromolecular catalytic process GO:0043832 | 23.08             | 13.30             | Motosi defective early emb WPPheno-type:0.001041 | 32.03           | 23.37           | WBPaper0039103_24hr, muscle enriched, WBBt:0006751_918 | 2.78           | 1.65           |
| 187            | F43H10.2-R53.4.21676 | R3 C4         | Organelle inner membrane GO:0019866 | 74.24             | 34.23             | Avoids bacterial lawn WPPheno-type:0.000402 | 27.00           | 6.57            | WBPaper0026900, intestine enriched, WBBt:0005772_1970 | 3.37           | 1.50           |
| 233            | his-46_859-his-64 | R10 C26       | DNA packaging complex GO:0044815 | 39.57             | 191.32            | Sister chromatid segregation defective early emb WPPheno-type:0.000772 | 26.48           | 94.19           | WBPaper0037950, germline-precursors, embryo_enriched, WBBt:0006849_974 | 0.00           | 0.07           |
| 93             | fdp-5-F17C11.2 | R4 C4          | Neuropeptide signaling pathway GO:0007218 | 56.65             | 67.09             | Sinusoidal movement variant WPPheno-type:0.000402 | 22.79           | 11.85           | WBPaper0037950, all-neurons larva enriched, WBBt:0003679_1013 | 22.28          | 3.93           |
| 2              | rps-5_2365-rpl-15_1635 | R10 C20       | Structural constituent of ribosome GO:0003735 | 24.02             | 68.88             | Plosetropic defects severe early emb WPPheno-type:0.000270 | 19.08           | 40.82           | WBPaper0037950, PVD-CELL-neuron,embryo_enriched, WBBt:0006831_876 | 0.00           | 0.23           |
| 143            | his-20_965-his-4 | R11 C22        | DNA packaging complex GO:0044815 | 30.10             | 191.32            | Sister chromatid segregation defective early emb WPPheno-type:0.000772 | 18.37           | 87.98           | WBPaper0024500, pharyngeal enriched, WBBt:0003681_329 | 0.00           | 0.18           |

Continued
Table 1. Most relevant coexpression cliques of the clique map, their size and position, GO-term assignment, phenotype enrichment and tissue enrichment.

| Clusternumber | Cluster name          | Clique position | Best GO-term                   | log10(pvalue) TEA | GO correction | Fold_TEA | Best TEA-Term | log10(pvalue) TEA | GO correction | Fold_TEA | Best TEA-Term | log10(pvalue) TEA | GO correction | Fold_TEA |
|---------------|----------------------|-----------------|-------------------------------|-------------------|---------------|---------|----------------|-------------------|---------------|---------|----------------|-------------------|---------------|---------|
| 96            | mup-2-unc-87_284     | R3 C13 Myofibril GO:0030016 | 31.32 27.72 Muscle system morphology variant WBPphenotype:0,001,063 | 17.34 8.57 WBPaper0031503.08_muscle_embryonews_WBbt_0003675_761 | 20.71 | 3.64 |
| 218           | H2BG01.5.1.1027_ H2BG01.5.1042 | R11 C24 Cell recognition GO:0008037 | 18.80 92.91 Axon fasciculation variant WBPphenotype:0,001,063 | 17.15 62.29 WBPaper0043521_Spermatogenic_WBbt_0005784_2743 | 0.00 | 0.33 |
| 42            | rab-28-jbts-14_9542  | R3 C5 Cell projection assembly GO:0030031 | 45.34 56.43 Amphiphosphatidylserinum morphology variant WBPphenotype:0,001,527 | 16.40 17.48 WBPaper0037590o_BAG_neuron_embryonews_WBbt_0006825_454 | 42.10 | 7.48 |
| 234           | pce-1-cyb-3.17196    | R7 C12 DNA replication GO:0006260 | 13.16 29.41 Cytokinesis variant WBPphenotype:0,001,063 | 16.17 11.19 WBPaper0037590o_pharyngeal-muscle_embryonews_WBbt_0005451_598 | 0.01 | 0.48 |
| 197           | unc-11.429-unc-11.430 | R12 C11 Phosphatidylinositol binding GO:0035991 | 15.76 172.19 Mid larval lethality WBPphenotype:0,001,116 | 15.12 160.50 WBPaper0037590o_cellosomeyotnex_embryonews_WBbt_0005751_570 | 0.09 | 0.76 |
| 1             | T40H4a.2-ZK1051.2    | R1 C6 Phosphores metabolic process GO:0006793 | 17.12 3.03 Spermatogenesis variant WBPphenotype:0,000,070 | 1.48 4.25 WBPaper0043521_Spermatogenic_WBbt_0005784_2743 | 165.27 | 4.57 |
| 20            | T22D3.1.5-ins-14     | R2 C1 Embryo development GO:0030790 | 13.09 1.97 Anionchannel WBPphenotype:0,001,082 | 11.50 6.39 WBPaper0057590o-germelinprecursors_embryonews_WBbt_0006849_974 | 73.49 | 5.82 |
| 273           | C01G10.14-dct-9.3227 | R2 C3 Regulation of cell shape GO:0008360 | 20.29 18.38 Spermatogenesis variant WBPphenotype:0,000,070 | 2.62 7.10 WBPaper0043521_Spermatogenic_WBbt_0005784_2743 | 71.95 | 3.62 |
| 111           | snb-9.22518.33D5.6.14044 | R2 C6 Cuticle development GO:0042335 | 16.79 11.64 Molvariant WBPphenotype:0,002,041 | 12.53 5.66 WBPaper0037590_o_hypofermum_embryonews_WBbt_0005753_734 | 56.15 | 6.08 |
| 90            | zdr-1-T09B9.3        | R1 C5 Intracompartment of membrane GO:0031224 | 52.94 1.97 Smooth endomembrane variant WBPphenotype:0,004,018 | 8.60 3.85 WBPaper0037590_o_allneuroslarv_enumbryonews_WBbt_0006879_1013 | 53.78 | 4.32 |
| 42            | rab-28-jbts-14_9542  | R3 C5 Cell projection assembly GO:0030031 | 45.34 56.43 Amphiphosphatidylserinum morphology variant WBPphenotype:0,001,527 | 16.40 17.48 WBPaper0037590o_BAG_neuron_embryonews_WBbt_0006825_454 | 42.10 | 7.48 |
| 213           | xhn-3.DDII1.5.20997 | R1 C12 Signaling GO:0023052 | 15.19 2.38 Backward point velocity increased WBPphenotype:0,002,325 | 7.12 8.29 WBPaper0037590_o_allneuroslarv_enumbryonews_WBbt_0006879_1013 | 41.49 | 4.53 |
| 155           | F42A9.7-T22B3.3      | R3 C11 Regulation of cell shape GO:0008360 | 9.67 17.73 Dauer metabolism variant WBPphenotype:0,001,547 | 1.51 2.28 WBPaper0043521_Spermatogenic_WBbt_0005784_2743 | 25.38 | 2.62 |
| 83            | sod-1-jnk-1.18695    | R1 C9 Nervous system development GO:0037399 | 21.52 4.87 Synaptic morphology variant WBPphenotype:0,000,616 | 10.59 8.04 WBPaper0031532_Larva_Pan_Neuronal_Enmbryonews_WBbt_0006879_1013 | 25.10 | 2.45 |
| 93            | flp-5-F17C11.2       | R4 C4 Neuropeptide signaling pathway GO:0007218 | 56.65 67.09 Smooth endomembrane variant WBPphenotype:0,004,018 | 22.79 11.85 WBPaper0037590_o_allneuroslarv_enumbryonews_WBbt_0006879_1013 | 22.28 | 3.93 |
| 96            | mup-2-unc-87_284     | R3 C13 Myofilament GO:0030016 | 31.32 27.72 Muscle system morphology variant WBPphenotype:0,001,063 | 17.34 8.57 WBPaper0031503.08_muscle_embryonews_WBbt_0003675_761 | 20.71 | 3.64 |
| 34            | dho-28.22199-acn-14  | R4 C9 Oxidative metabolism GO:0043436 | 14.25 9.18 Lipid metabolism variant WBPphenotype:0,000,070 | 4.43 3.34 WBPaper0037590_intestine_embryonews_WBbt_0005752_866 | 20.41 | 3.78 |

Gonad development in agreement with the sterility phenotype observed. Comparing the three experiments a weak correlation can be found between experiment 2 and 1 (R² = 0.148) on a gene-by-gene level, which is increased, if cliques are compared (R² = 0.307, Fig. 1c). The same trend can be seen between experiments 3 and 1 correlating with R² = 0.622 for a gene-to-gene comparison, which increases to R² = 0.764, if cliques are compared (Fig. 1d). Moreover the significance analysis employing 20 random cliques shows that the most strongly up- and down-regulated cliques also are usually fulfilling the 1e-5 significance criterium in the compared experiments (Fig. 1c and d, colored in red).
**unc-45 RNAi leads to delayed conclusion of sperm and vulva development.** We then investigated the RNAi treatment against the HSP-90 cofactor *unc-45* with the same approach. *unc-45* RNAi-treatment leads to developmental disruptions and incomplete fertility at a more adult stage. To see, whether differences in the cliques can be observed we performed two independent RNAi-experiments with subsequent transcriptome analysis on DNA microarrays. Analysis with TAC showed a weaker response compared to the *hsp-90* RNAi in both experiments (Fig. 2a, Supplemental Fig. 2a). This also was evident in the analysis of the 307 expression cliques, where the color scheme had to be adjusted to visualize the concerted reactions (Fig. 2b and Supplemental Fig. 2b). Gene–gene comparisons showed a coefficient of determination of 0.15 between the experiments. When cliques were compared a coefficient of determination of 0.48 was obtained (Fig. 2c), confirming that also very weak responses can yield higher levels of repeatability by comparing matched groups of genes and not individual genes.

Like with *hsp-90* RNAi, specific cliques were found in all experiments to be significantly altered in their expression behavior. Upregulated are a few smaller cliques, like col-117-col-167_1015, msp-63-msp-33 and abu-7-abu-8_22491. These represent decisions to produce cuticle collagens, linker cell movement and induction behavior. Upregulated are a few smaller cliques, like col-117-col-167_1015, msp-63-msp-33 and individual genes. Very weak responses can yield higher levels of repeatability by comparing matched groups of genes and not individual genes.

Expression in developmental stages is altered in similarity to the RNAi-induced arrest. Having observed cliques with altered expression, we aimed at understanding, whether these expression changes are specific for one developmental transition occurring at the time point of arrest. We thus generated a time series of development ranging from embryo to late adult and compared the expression of all 307 cliques and in particular of those found relevant for *unc-45* RNAi.

Striking differences were observed, when comparing the stages of each series (Supplemental Fig. 3), while differences between experiments of the same stage were small (Supplemental Figs. 4 and 5). Interestingly, also in these comparisons most of the isolated expression cliques showed coordinated expression differences, and also strong responses could be observed for the later developmental stages (Supplemental Fig. 6). In total more then 80% of the cliques show a statistically significant expression change during the development from embryo to 16 day adult and this also relates to most cliques found affected after *unc-45* RNAi (Fig. 2d, 2e and 2f, their development). While only few cliques were affected upon *unc-45* RNAi treatment, *hsp-90* RNAi is expected to yield a much stronger response.

Indeed a drop is observed in the expression of most upregulated cliques between L4 and day6 adult. In these cases the developmental delay may be the reason of the observed higher expression. A opposite pattern is observed for the downregulated gene cliques, with the exception of two cliques, which are not appropriately regulated: T05E12.6_1239-T05E12.6_12396 and gpd-3_977-aldo-1_21168, both of which appear to regulate metabolism.

Expression in developmental stages is altered in similarity to the *hsp-90* RNAi-induced arrest. We next tested, whether also for the *hsp-90* RNAi-treated nematodes developmental stages can be defined. The complexity of the differential expression between *hsp-90* RNAi arrested nematodes and young adults allow to compare the obtained expression patterns with known patterns from larval development. We thus were interested to see, whether the full extent of the transcriptional changes can be explained by the observed developmental delay. Therefore we utilized publicly available microarray experiments on nematode development to help identify transcriptional units in the clique map that report on comparable steps during development to help identify transcriptional units in the clique map that report on comparable steps during development.
Figure 1d.
| Cluster name | Clique position | Best GO-Term | Best PEA-Term | Best TEA-Term | Mean STD | Log10(p) Exp 1 | Log10(p) Exp 2 | Log10(p) Exp 3 |
|--------------|----------------|--------------|--------------|--------------|----------|----------------|----------------|----------------|
| col-138-col-49 | R9 C23 | Structural constituent of cuticle GO:0042302 | Blistered WBPhe- notype:0000255 | WBPaper00037950_ -coelomocytes_ larva/enriched_ WBBt_0003751_229 | 1.43 ± 0.71 | 28.5 | 35.6 | 70.2 |
| abu-7-abu-8_22491 | R8 C26 | Response to unfolded protein GO:0006986 | Dauer constitutive WBPhe- notype:0000102 | WBPaper00024505_ -pharyngal/enriched_ WBBt_0003681_329 | 1.43 ± 0.66 | 38.9 | 38.4 | 51.4 |
| agmo-1_5527-F53B1.4 | R10 C14 | Pyridoxal phosphate binding GO:00030170 | Molt variant WBPhe- notype:0002041 | WBPaper00037950_ -hypodermis/-embryo/enriched_ WBBt_0005733_734 | 1.29 ± 0.76 | 14.4 | 3.2 | 48.0 |
| bus-8_3160-K04H4.2_2324 | R9 C9 | Amino sugar metabolic process GO:0006040 | Molt variant WBPhe- notype:0002041 | WBPaper00024505_ -pharyngal/enriched_ WBBt_0003681_329 | 1.25 ± 0.73 | 30.4 | 3.5 | 103.9 |
| R12E2.14_75-R12E2.15 | R6 C9 | Structural constituent of cuticle GO:0042302 | Dumpy WBPhe- nototype:0009,583 | WBPaper00037950_ -germline-precursors/-embryo/enriched_ WBBt_0006849_974 | 1.22 ± 0.67 | 51.2 | 21.0 | 182.3 |
| mlt-9_22518-F33D4.6_14044 | R2 C6 | Cuticle unfolded protein GO:0042335 | Molt variant WBPhe- notype:0002041 | WBPaper00037950_ -hypodermis/-embryo/enriched_ WBBt_0005733_734 | 1.08 ± 0.72 | 137.7 | 2.6 | 133.0 |
| R12A1.3-M195.2 | R6 C19 | Amino sugar metabolic process GO:0006040 | Dauer constitutive WBPhe- notype:0000102 | WBPaper00024505_ -pharyngal/enriched_ WBBt_0003681_329 | 1.05 ± 0.63 | 42.2 | 14.3 | 46.7 |
| hsp-16.2-F44E5.4_19238 | R12 C4 | Response to heat GO:0009408 | cadmium response variant WBPhe- notype:0016535 | WBPaper00037950_ -coelomocytes/-embryo/enriched_ WBBt_0005731_229 | 1.03 ± 0.62 | 3.6 | 6.2 | 1.7 |
| lys-3-tp-1 | R11 C40 | Carbohydrate metabolic process GO:0005975 | Male nervous system development variant WBPhe- notype:0001088 | WBPaper00040420_ALM-PLM/enriched_ WBBt_0005406_198 | 0.99 ± 0.25 | 6.4 | 28.8 | 6.0 |
| col-117-col-167_1015 | R9 C25 | Structural constituent of cuticle GO:0042302 | Dumpy WBPhe- nototype:0005835 | WBPaper00037950_ -germline-precursors/-embryo/enriched_ WBBt_0006849_974 | 0.94 ± 0.47 | 10.6 | 10.3 | 20.0 |
| C38C6.3-acdh-6 | R6 C14 | Intrinsic component of membrane GO:00311224 | Intestinal vacuole WBPhe- notype:0004128 | WBPaper00037950_ -hypodermis/-embryo/enriched_ WBBt_0005733_734 | 0.91 ± 0.59 | 71.0 | 1.4 | 59.3 |
| pnp-54-abu-9 | R6 C11 | Response to stress GO:0006986 | Shortened life span WBPhe- notype:0001171 | WBPaper00024505_ -pharyngal/enriched_ WBBt_0003681_329 | 0.91 ± 0.4 | 37.6 | 43.3 | 33.7 |
| col-146-col-133 | R9 C28 | Structural constituent of cuticle GO:0042302 | Dumpy WBPhe- nototype:0005835 | WBPaper00037950_ -coelomocytes/-embryo/enriched_ WBBt_0002751_229 | 0.89 ± 0.46 | 10.0 | 6.5 | 26.7 |
| C36C5.12-F57G8.7 | R11 C9 | Negative regulation of proteolysis GO:0045861 | Male tail morphology variant WBPhe- notype:0000870 | WBPaper00037950_ -hypodermis/-embryo/enriched_ WBBt_0005731_229 | 0.83 ± 0.71 | 13.9 | 1.3 | 5.0 |
| col-128-cdh-10_9234 | R3 C12 | Peptidase activity GO:0008233 | Molt variant WBPhe- notype:0002041 | WBPaper00037950_ -hypodermis/-embryo/enriched_ WBBt_0005733_1250 | 0.82 ± 0.56 | 66.1 | 0.6 | 61.0 |
| ptr-23_236.ptr-23_16340 | R12 C6 | Male sex differentiation GO:0046661 | Developmental pigmentation variant WBPhe- notype:0001099 | WBPaper00037950_ -hypodermis/-embryo/enriched_ WBBt_0005733_734 | 0.79 ± 0.56 | 6.0 | 0.2 | 9.5 |
| C18C5.2-Y58A7A.3 | R4 C11 | Immune system process GO:0002376 | Cadmium response variant WBPhe- notype:0001653 | WBPaper00037950_ -coelomocytes/-embryo/enriched_ WBBt_0002751_229 | 0.77 ± 0.31 | 36.6 | 83.0 | 39.9 |
| sqt-2-dpy-9 | R9 C6 | Structural constituent of cuticle GO:0042302 | Dumpy WBPhe- nototype:0005835 | WBPaper00037950_ -pharyngal/enriched_ WBBt_0005733_1250 | 0.76 ± 0.48 | 13.0 | 1.0 | 18.9 |
| dos-2-grd-2 | R8 C7 | Extracellular region GO:0003576 | Pericellular component morphology variant WBPhe- notype:0009112 | WBPaper00037950_ -hypodermis/-embryo/enriched_ WBBt_0005733_734 | 0.75 ± 0.52 | 15.4 | 0.3 | 36.4 |

Continued
| Cluster name         | Clique position | Best GO-Term                          | Best PEA-Term                          | Best TEA-Term                          | Mean STD     | Log10(p) Exp 1 | Log10(p) Exp 2 | Log10(p) Exp 3 |
|----------------------|-----------------|---------------------------------------|---------------------------------------|---------------------------------------|--------------|---------------|---------------|---------------|
| K08D10.9-F46A8.1     | R11 C42         | Immune system process GO:002576       | Actin organization biogenesis variant WBPonymyte:0001587 | WBPpaper0037950_ectrocyt-cell larva_enriched_ WBBt_0005812_528 | 0.72 ± 0.12  | 3.7           | 12.9          | 2.2           |
| vit-2-vit-4_22519     | R12 C22         | Extracellular region GO:0005576       | Pathogen susceptibility increased WBPonymyte:0001013 | WBPpaper0037950 phosphorylase-muscle_embryo_enriched_ WBBt_0005451_598 | −1.57 ± 1.42 | 47.9          | 2.5           | 5.7           |
| C17E7.4-T06D4.1      | R5 C13          | ribonuclease-protein granule GO:0035770 | P granule defective WBPonymyte:0001301 | WBPpaper0037950 phosphorylase-muscle_embryo_enriched_ WBBt_0005451_598 | −1.07 ± 0.87 | 168.7         | 1.2           | 72.1          |
| 171971_x_at-D1054.11_184 | R11 C37       | Cell GO:0005623                       | Egg laying defective WBPonymyte:0000006 | WBPpaper0037950 hypodermis-larva_enriched_ WBBt_0005713_1250 | −0.96 ± 0.84 | 1.4           | 0.7           | 0.6           |
| sea-1-R04D3.4        | R7 C13          | Nucleoside-triphosphate regulator activity GO:0060589 | Embryonic development variant WBPonymyte:000749 | WBPpaper0037950 GABAergic-motor-neurons_larva_enriched_ WBBt_0005190_132 | −0.93 ± 0.69 | 39.7          | 4.0           | 53.7          |
| T24D1.3-egg-1        | R7 C17          | Embryo development GO:0009790         | Polar body defective early emb WBPonymyte:0001147 | WBPpaper0037950 GABAergic-motor-neurons_larva_enriched_ WBBt_0005190_361 | −0.82 ± 0.72 | 36.0          | 0.3           | 24.0          |
| C46C2.5_15926-W03F11.1 | R9 C22         | Carbohydrate binding GO:0030246       | Apoptosis increased WBPonymyte:000183 | WBPpaper0037950 GABAergic-motor-neurons_larva_enriched_ WBBt_0005190_361 | −0.82 ± 0.57 | 15.7          | 2.5           | 13.8          |
| ZC373.2-Y62HA9.6_1596 | R3 C18         | Flavonoid metabolic process GO:0009812 | Cell membrane organization biogenesis variant WBPonymyte:0001982 | WBPpaper0037950 dopaminergic-neurons_larva_enriched_ WBBt_0006746_1230 | −0.8 ± 0.7   | 53.5          | 6.0           | 32.5          |
| ZK1053.4-C08F1.6     | R4 C16          | Embryo development GO:0009790         | Embryonic development variant WBPonymyte:000749 | WBPpaper0037950 hypodermis-larva_enriched_ WBBt_0005733_734 | −0.73 ± 0.4  | 70.9          | 12.9          | 40.5          |
| TUSE12.6_12439-TUSE12.6_12396 | R10 C3    | Lipid catabolic process GO:0016042    | Transgene expression increased WBPonymyte:0001236 | WBPpaper0037950 phosphorylase-muscle_embryo_enriched_ WBBt_0005451_598 | −0.72 ± 0.82 | 28.7          | 2.8           | 3.3           |
| fbx-28-sde-28        | R7 C8           | Modification-dependent macromolecule catabolic process GO:0043632 | L1 larval development variant WBPonymyte:000751 | WBPpaper0037950 phosphorylase-muscle_embryo_enriched_ WBBt_0005733_734 | −0.69 ± 0.39 | 26.3          | 23.5          | 41.6          |
| K09D9.12-T10C6.10    | R10 C7          | Protein poly-ubiquitination GO:0060209 | Fat content reduced WBPonymyte:0001183 | WBPpaper0037950 germline-preursors_embryo_enriched_ WBBt_0006849_974 | −0.62 ± 0.54 | 7.6           | 1.2           | 3.8           |
| puf-3-oma-2_18268    | R5 C14          | Reproduction GO:0000003                | Meiotic chromosome segregation variant WBPonymyte:0001499 | WBPaper0037950 germline-preursors_embryo_enriched_ WBBt_0006849_974 | −0.6 ± 0.53  | 24.0          | 0.8           | 12.3          |
| 172276_x_at-Y116F11B.10_466 | R12 C20       | Chromosome segregation GO:0007059     | Rachis wide WBPonymyte:0001943 | WBPaper0036375 enriched_in_ PVU_OLL_ WBBt_0006831_2180 | −0.59 ± 0.47 | 1.2           | 0.2           | 1.0           |
| C41G7.3_2766-ani-2_2946 | R8 C2           | Multi-organism reproductive process GO:0044703 | Cytokinesis variant WBPonymyte:0002408 | WBPaper0037950 germline-preursors_embryo_enriched_ WBBt_0006849_974 | −0.59 ± 0.51 | 26.0          | 0.8           | 9.0           |
| T22D1.5-imx-14       | R2 C1           | Embryo development GO:0009790         | Aneuploidy WBPonymyte:0001882 | WBPaper0037950 germline-preursors_embryo_enriched_ WBBt_0006849_974 | −0.54 ± 0.48 | 122.7         | 2.0           | 60.1          |
| let-99_22121-B0238.9_11154 | R6 C21         | Organelle fusion GO:0048285          | Embryonic development variant WBPonymyte:000749 | WBPaper0037950 germline-preursors_embryo_enriched_ WBBt_0006849_974 | −0.52 ± 0.44 | 16.6          | 0.2           | 15.4          |
| Y116A8C.19-F38C2.7   | R10 C25         | Poly(A) RNA binding GO:0048822       | Dauer metabolism variant WBPonymyte:0001547 | WBPaper0040420 FLP_enriched_ WBBt_0006828_288 | −0.49 ± 0.25 | 4.8           | 5.5           | 4.5           |

Continued
development. We employed microarray data from three experimental series (Table 4) and initially compared developmental transitions, showing similarity to the differences we observe in the RNAi-treated nematodes. These comparisons were L3/young adult, L4/young adult and L4let/young adult (Fig. 3a–c) as investigated in GSE46288/GSE4628928. Clearly similarities can be observed between the hsp-90 RNAi treated nematodes and the L4 larvae, when each of them is compared to the young adult control. In fact, most of the cliques correlate in color and correlation analysis shows a coefficient of determination with these data of 0.4046, 0.5913 and 0.5915 (Fig. 3d). Based on these values, hsp-90 RNAi-arrested nematodes best correspond to a L4-larval like state. Only few clear differences can be observed compared to L4 or L4-lethargus, while several cliques deviate from L3-like state. Judged from the few differences to L4 state, it might be that the chronological timing of the events during development is misaligned in hsp-90 RNAi-arrested nematodes.

We further investigated, whether the expression behavior matches the known expression behavior during development and aging. To this end we used the information gained previously that a fraction of the misregulated genes are daf-16 targets11. We tested, which of the cliques from the clique map contain daf-16 targets and then tested, whether those are regulated in coordance with developmental progress. Indeed, targets upregulated and suppressed by DAF-16 are enriched in several cliques (the 15 most prominent shown in Table 5, more information in Fig. 4a and b). Comparing the clusters identified in Eckl et al. (2017), with the current cliques we also observe a clear enrichment among several of the 307 cliques (Table 5). As speculated in Eckl et al., among the cluster “Up1” there are many genes, which are regulated by DAF-16, while cluster “Up2” does not enrich daf-16 targets (Table 5). Mapping all cliques onto the network developed in Eckl et al. the enrichment of these cliques in certain parts of the network becomes evident. For the downregulated genes, also DAF-16 enriching cliques are among those containing these genes11. Therefore, especially among the upregulated genes, cliques are present, which contain an elevated level of daf-16 target genes.

Interestingly, these cliques are upregulated despite their developmental program, which aims for downregulation. Thus, the presence of these cliques suggests a simultaneous modification to the dauer-program outside the developmental program after hsp-90 RNAi induced growth arrest.

| Cluster name | Clique position | Best GO-Term | Best PEA-Term | Best TEA-Term | Mean STD | Log10(p) Exp 1 | Log10(p) Exp 2 | Log10(p) Exp 3 |
|--------------|----------------|--------------|---------------|--------------|----------|----------------|----------------|----------------|
| daf-18_2911-ced-2_4092 | R8 C23 | Nuclear transport GO:00051169 | Cell death variant WBP0000729 | WBPaper0000750_ GABAergic-motor-neurons_, larva_enriched_, WBBb_005190_132 | -0.48 ± 0.4 | 6.9 | 0.6 | 10.0 |
| pcn-1-cyb-3_17196 | R7 C12 | DNA replication GO:0006260 | Cytokinesis variant WBP0002408 | WBPaper0000750_ pharyngeal-muscle_, embryo_enriched_, WBBb_005451_598 | -0.47 ± 0.38 | 17.2 | 1.5 | 5.3 |
| C10C5.3-C10C5.5 | R12 C40 | Oxoacid metabolic process GO:0043436 | Dauer constitutive WBP0000812 | WBPaper0000750_ pharyngeal-muscle_, embryo_enriched_, WBBb_005451_598 | -0.47 ± 0.48 | 2.6 | 5.1 | 2.2 |

Table 2. Most strongly affected cliques by hsp-90 RNAi and their characteristics. Clique positions (letter = row, number = position from left to right) correspond to the clique map shown in Fig. 1b.
Discussion
In this study, we analysed microarray data from C. elegans based on preformed coregulated expression cliques. This approach has been applied successfully in the yeast model organism by us\(^7\), but the applicability of this method to multicellular organisms has not been clear. We thus used the algorithms developed for yeast to also generate high quality coexpression cliques from nematode expression data and then validated them by GO-term enrichment, phenotype enrichment and tissue enrichment and by selective clique responses in individual microarray experiments. Based on our data from the developmental process of C. elegans, we believe that this analysis method could have broader use in the analysis of gene expression data from nematodes. This is evident from the correlated responses of cliques during nematode developmental transitions.

Recently also a different approach was reported to utilize genome-wide co-expression cliques for C. elegans\(^9\). In contrast to our approach, in this study an individual gene could be assigned to multiple cliques (on average 3) and also negative correlation was included. This makes the construction of a static clique map as used by us more difficult, but may include details missed by our approach. Both approaches will have their advantages. In the method described by us, we focus on the strongest connection and blank out those that might be secondary based on numbers, but still achieve very high levels of correlation with GO-terms, phenotypes and tissue specificity for most of the cliques.

One way to use the cliques could be by employing the popular GSEA platform\(^{30}\), where our cliques can be either used as a single input file covering the whole genome or as part of the global collection of gene sets. Another way to use the cliques can also be via the clusterEX.de webserver that we have set up and will further develop for the purpose of gene expression analysis based on known co-expression relationships. It therefore will be interesting to see, how further useful applications will be developed based on these predefined gene sets.

Integrating unc-45 into the developmental time line exposes distinct cliques for developmental stop. We first analysed unc-45 depleted nematodes. In these nematodes, the depletion of unc-45 leads to developmental arrest and paralysis in almost adult animals. Here the comparison with the young adult nematode shows that certain cliques are misregulated and some of those cliques also represent developmental marker cliques as suggested by our evaluation procedure. These marker genes help to map the developmental status of the unc-45 depleted organisms. Clearly unc-45 depleted nematodes are close to N2 nematodes in this approach, but defined changes in certain genes help to map the events that did not unfold during development.

To evaluate the disruption of vulva development, we individually tested the genes transcriptionally regulated during this process and their specific regulation (Table 3): eff-1 (log2(dExp) = 0.185), egl-18 (−0.035), egl-17 (0.000), lin-3 (−0.015), lin-31 (0.00), lin-39 (0.00), egl-30 (0.02), lag-2 (−0.09), apx-1 (0.055), dsl-1 (−0.085 as part of fbxc-28-sdz-28) and elt-6 (-0.065), all of which are getting induced during vulva development. In a critical step during vulva development the VPCs express LIN-39, which together with its cofactors CEH-20 and UNC-62, activates the expression of ref-2, which inhibits the expression of the fusogen EFF-1\(^{33}\). In UNC-45 depleted nematodes, ref-2 is not yet upregulated compared to mock treated nematodes (−0.675 and resides in clique cfz-2_18944-cfz-2_2268, which is downregulated twice significantly, but not very strongly) and also ref-1 is lower expressed in unc-45 RNAi-treated nematodes (−0.46, ZK1053.4-C08F1.6), even though lin-39 is expressed as in the control and eff-1 is higher expressed (0.185, tnt-4-myo-1_2160), as expected for vulva development. Thus, based on these expression patterns the induction to generate the vulva is not transmitted properly by the anchor cell from the developing gonad. Also lin-12 (−0.31, sol-1-jnk-1_18695), cwn-1 (−0.26, chd-7_16664-jmj-d-3.1) and vnu-g-1 (−0.175, nrde-3-tra-4_10484) are downregulated, further implying that central decisions to induce the vulva have not been made yet.

Regarding the germline, asb-2 is reduced (−0.21, tars-1-AFFX-r2-3026-5_at) and the nspd-proteins are still upregulated together with msp-proteins (Fig. 2d\(^{34}\)), implying that sperm development is not completed yet, while the expression of the upstream regulators spn-4 and neg-1\(^{35}\) is at the same level as in the normally developed adult. Also the regulators of msp-expression set-17 and csr-1 are expressed at the level of the control nematodes\(^{36}\), implying that sperm-development is almost finished\(^{37\}, 38\).

Integrating hsp-90 into the time line data exposes defined clusters for developmental stop. We used this clique map to also analyse the depletion of hsp-90. While depletion of hsp-90 leads to developmental arrest and reduced motility in late larval stages, it also leads to defined transcriptional changes. To analyse the causes, we performed microarray experiments under wildtype conditions and under conditions, where the chaperone is depleted. Based on the clique analysis, it is obvious that certain developmental milestones are not reached yet in the HSP-90 depleted animals. Based on this analysis these nemtodes arrest in a late larval stage with additional misregulation of DAF-16 target genes.
Previously it had been observed that the Top300 genes from the hsp-90 RNAi analysis showed partial overlap with daf-16 regulated genes. We thus employed the gene-list from this previous study to identify the cliques, which now represent these genes. Indeed the correlation is fairly clear, with the cliques C17H1.6-C17H1.13, C32F10.4-D1086.2 and C10C5.2-Y58A7A.3 being mostly overlapping with the previous cluster1_up and the cliques col-138-col-49, R12E2.14_75-R12E2.15, mlt-9_22518-F33D4.6_14044 being mostly overlapping with the cluster2_up. Utilizing the ranked list of daf-16 target genes, we also determined which cliques most strongly are enriched in the Top750 and Bottom750 of this ranked list. These cliques are found mostly in cluster1_up confirming that the identification of this correlation also is visible from the clique map. Interestingly these cliques represent those that are differently regulated compared to the L4 larval stage. Thus the HSP-90 depletion leads to higher expression levels in a daf-16 regulated cluster (cluster1_up) and a daf-16 independent cluster (cluster2_up). With the daf-16 independent cluster containing mostly cliques related to larval development, apparently the depletion of HSP-90 induces both of these processes. Whether they are connected via secondary effects is unclear to date, especially as the developmental timing of DAF-16 activity is a well described phenomenon.

Thus, based on several clearly regulated marker cliques, hsp-90 arrested nematodes, like unc-45 arrested nematodes, can be positioned in respect to a developmental time axis.
| Cluster name | Clique position | Best GO-Term | Best PEA-Term | Best TEA-Term | Mean STD | Log10(p) Exp 1 | Log10(p) Exp 2 |
|--------------|----------------|-------------|--------------|--------------|----------|--------------|--------------|
| nspd-1-nspd-2 | R12 C1         | Structural constituent of cuticle GO:0042302 | Dumpy WBphenotype:0000583 | WPaper00037950_ coelomocytes_embryo_enriched_WBbt_0005751_570 | 0.38 ± 0.12 | 4.41 | 1.84 |
| msp-36-msp-55 | R10 C19        | Lipid storage GO:0019915 | Linker cell migration variant WBphenotype:0001511 | WPaper00040420_FLP_enriched_WBbt_0006828_288 | 0.35 ± 0.08 | 1.15 | 2.54 |
| msp-63-msp-33 | R9 C14         | Lipid storage GO:0019915 | Linker cell migration variant WBphenotype:0001511 | WPaper00040420_FLP_enriched_WBbt_0006828_288 | 0.31 ± 0.09 | 9.72 | 9.08 |
| hsp-16.2-F44E5.4_19238 | R12 C4 | Response to heat GO:0019915 | Cadmium response variant WBphenotype:0001653 | WPaper00037950_ coelomocytes_larva_enriched_WBbt_0005751_229 | 0.29 ± 0.17 | 16.49 | 1.60 |
| abu-7-abu-8_22491 | R8 C26 | Response to unfolded protein GO:0006986 | Dauer constitutive WBphenotype:0000012 | WPaper00024505_pharyngeal_embryo_enriched_WBbt_0003681_329 | 0.25 ± 0.08 | 63.31 | 5.15 |
| col-138-col-49 | R9 C23         | Structural constituent of cuticle GO:0042302 | Blistered WBphenotype:0000025 | WPaper00037950_ coelomocytes_larva_enriched_WBbt_0005751_229 | 0.24 ± 0.22 | 34.90 | 0.93 |
| col-117-col-167_1015 | R9 C25 | Structural constituent of cuticle GO:0042302 | Blistered WBphenotype:0000025 | WPaper00037950_ germline-precursors_embryo_enriched_WBbt_0006849_974 | 0.20 ± 0.06 | 29.40 | 5.29 |
| pqn-54-abu-9 | R6 C11         | Response to unfolded protein GO:0006986 | Shortened life span WBphenotype:0001171 | WPaper00024505_pharyngeal_embryo_enriched_WBbt_0003681_329 | 0.2 ± 0.03 | 43.57 | 13.10 |
| R12E2.14_75-R12E2.15 | R6 C9 | Structural constituent of cuticle GO:0042302 | Dumpy WBphenotype:0000583 | WPaper00037950_ germline-precursors_embryo_enriched_WBbt_0006849_974 | 0.18 ± 0.08 | 47.12 | 8.99 |
| lys-3-tsp-1 | R11 C40        | Carbohydrate metabolic process GO:0039575 | Male nervous system development variant WBphenotype:0001008 | WPaper00040420_ALM_PLM_enriched_WBbt_0005406_198 | 0.14 ± 0.22 | 27.93 | 1.06 |
| C10C5.3-C10C5.5 | R12 C40       | Oxoacid metabolic process GO:0043436 | Dauer constitutive WBphenotype:0000012 | WPaper00037950_pharyngeal-muscle_embryo_enriched_WBbt_0005451_598 | 0.17 ± 0.05 | 4.43 | 2.28 |
| F07A5.2-R10E9.2 | R5 C7          | Sodium ion transport GO:0006814 | Nicotine response variant WBphenotype:0001573 | WPaper00045521_Spermatogonic_WBbt_0005784_2743 | 0.15 ± 0.04 | 6.82 | 34.38 |
| K11C4.1_20445-mib-1.1 | R8 C22       | Regulation of cell shape GO:0008360 | Fat content increased WBphenotype:0001184 | WPaper00045521_Spermatogonic_WBbt_0005784_2743 | 0.14 ± 0.03 | 6.02 | 8.77 |
| sqs-2_16507-sqs-3_1032 | R12 C27     | Response to hormone GO:0009725 | Movement variant WBphenotype:0001206 | WPaper00026980_intestine_enriched_WBbt_0005772_1970 | 0.13 ± 0.0 | 1.96 | 2.06 |
| col-146-col-133 | R9 C28         | Structural constituent of cuticle GO:0042302 | Dumpy WBphenotype:0000583 | WPaper00037950_coelomocytes_larva_enriched_WBbt_0005751_229 | 0.12 ± 0.02 | 11.00 | 2.77 |
| F42A9.7-T22B3.3 | R3 C11        | Regulation of cell shape GO:0008360 | Dauer metabolism variant WBphenotype:0001547 | WPaper00045521_Spermatogonic_WBbt_0005784_2743 | 0.11 ± 0.03 | 7.97 | 29.43 |
| bus-8_3160-K04H4.2_2324 | R9 C2         | Amino sugar metabolic process GO:0006040 | Molt variant WBphenotype:0002041 | WPaper00024505_pharyngeal_embryo_enriched_WBbt_0003681_329 | 0.11 ± 0.08 | 8.94 | 0.92 |
| R12A13.1-M1952 | R6 C19         | Amino sugar metabolic process GO:0006040 | Dauer constitutive WBphenotype:0000012 | WPaper00024505_pharyngeal_embryo_enriched_WBbt_0003681_329 | 0.1 ± 0.04 | 6.65 | 3.63 |
| T28A11.5-T06C12.14 | R12 C14       | Extracellular region GO:0005576 | Dumpy WBphenotype:0000583 | WPaper00037950_excretory-cell_larva_enriched_WBbt_0005812_528 | 0.1 ± 0.05 | 0.71 | 2.01 |
| agmo-1_5527-F53B1.4 | R10 C14       | Pyridoxal phosphate binding GO:0303170 | Molt variant WBphenotype:0002041 | WPaper00037950_hypodermis_embryo_enriched_WBbt_0005733_734 | 0.1 ± 0.11 | 7.44 | 0.70 |
| 171971_x-at-D10541.11_184 | R11 C37     | Cell GO:0005623 | Egg laying defective WBphenotype:0000006 | WPaper00037950_hypodermis_larva_enriched_WBbt_0005733_1250 | − 0.55 ± 0.09 | 3.70 | 0.66 |
| gpd-3_977-aldo-1_21168 | R9 C26        | Glycosyl compound metabolic process GO:1901657 | Fat content reduced WBphenotype:0001183 | WPaper00031002_24hr_muscle_enriched_WBbt_0003675_918 | − 0.51 ± 0.01 | 9.02 | 4.52 |
| T05E12.6_12439-T05E12.6_12396 | R10 C3   | Lipid catabolic process GO:0016042 | Transgene expression increased WBphenotype:0001236 | WPaper00037950_pharyngeal-muscle_embryo_enriched_WBbt_0005451_598 | − 0.29 ± 0.15 | 60.37 | 3.63 |

Continued
| Cluster name   | Clique position | Best GO-Term | Best PEA-Term | Best TEA-Term | Mean STD | Log10(p) Exp 1 | Log10(p) Exp 2 |
|---------------|-----------------|--------------|---------------|---------------|----------|---------------|---------------|
| ZK1053.4-C08F1.6 | R4 C16          | Embryo development GO:0009790 | Embryonic development variant WBPhenotype:0000749 | WBPaper0037950_ hypodermis_embryos_enriched_, WBbt_0005190_361 | −0.26±0.12 | 26.51         | 73.26         |
| C46C2.5_15926-W03F11.1 | R9 C22         | Carbohydrate binding GO:0030246 | Apoptosis increased WBPhenotype:0000183 | WBPaper0037950_ GABAergic-motor-neurons_embryos_enriched_, WBbt_0005190_361 | −0.24±0.02 | 8.57          | 10.00         |
| ZC373.2-Y62H9A.6_1596 | R3 C18         | Flavonoid metabolic process GO:0009812 | Cell membrane organization biogenesis variant WBPhenotype:0001982 | WBPaper0037950_ dopaminergic-neurons_larva_enriched_, WBbt_0005733_734 | −0.22±0.03 | 46.68         | 19.54         |
| nspc-1_614-nspc-10_22525 | R10 C12       | Extracellular region GO:0005576 | Spermatogenesis variant WBPhenotype:0000183 | WBPaper0037950_ intestine_larva_enriched_, WBbt_0005733_734 | −0.19±0   | 9.53          | 10.38         |
| C46C2.5_15925-F17E9.2 | R10 C8          | Hydrolase activity—acting on glycosyl bonds GO:0016798 | Embryonic development variant WBPhenotype:0000749 | WBPaper0037950_ hypodermis_embryos_enriched_, WBbt_0005190_361 | −0.2±0.01 | 6.65          | 6.25          |
| C03B1.14-F46C3.2 | R8 C21          | Membrane GO:0016020 | Chemical hypersensitive WBPhenotype:0001918 | WBPaper0037950_ intestine_larva_enriched_, WBbt_0005733_734 | −0.18±0.08 | 10.65         | 27.90         |
| fbxb-28-sdz-28 | R7 C8           | Modification-dependent macromolecule catabolic process GO:0043632 | L1 larval development variant WBPhenotype:0000751 | WBPaper0037950_ hypodermis_embryos_enriched_, WBbt_0005190_361 | −0.17±0.1 | 1.20          | 13.08         |
| fbxb-13-fbxb-24 | R8 C18          | Protein oligomerization GO:0051259 | Cholinergic agonist resistant WBPhenotype:0001578 | WBPaper0024505_ pharyngeal_neuron_enriched_, WBbt_0005190_361 | −0.14±0.07 | 2.78          | 9.72          |
| dsh-1_3575-C40A11.4 | R5 C17         | Protein oligomerization GO:0051259 | Ectopic expression transgene WBPhenotype:0001276 | WBPaper0037950_ hypodermis_embryos_enriched_, WBbt_0005733_734 | −0.14±0.01 | 2.03          | 0.89          |
| vem-1-ugt-58 | R12 C35         | Oxoacid metabolic process GO:0043436 | Epithelial cell physiology variant WBPhenotype:0000986 | WBPaper0037950_ hypodermis_embryos_enriched_, WBbt_0005190_361 | −1.04±0.09 | 2.82          | 41.16         |
| sdz-10-fbxb-62 | R3 C17          | Glycosylation GO:0070085 | L1 larval development variant WBPhenotype:0000751 | WBPaper0037950_ hypodermis_embryos_enriched_, WBbt_0005190_361 | −0.12±0.03 | 0.84          | 0.77          |
| Y41D4B.17-K10D11.6 | R12 C32        | Immune system process GO:0002376 | Explored through vulva WBPhenotype:0000838 | WBPaper0037950_ hypodermis_embryos_enriched_, WBbt_0005190_361 | −0.12±0.09 | 0.64          | 4.30          |
| fbxb-31-fbxb-119 | R11 C28         | Embryos development GO:0009790 | Transgene expression reduced WBPhenotype:0000136 | WBPaper0037950_ hypodermis_embryos_enriched_, WBbt_0005190_361 | −0.12±0.09 | 0.98          | 2.14          |
| R03E1.2_7363-ucr-2.1 | R10 C30       | Mitochondrion GO:0005739 | mRNA levels increased WBPhenotype:0000136 | WBPaper0037950_ hypodermis_embryos_enriched_, WBbt_0005190_361 | −0.12±0.03 | 0.98          | 2.14          |
| C35C5.8_15869-best-1 | R10 C21        | Transmembrane transport GO:0055085 | Fat content increased WBPhenotype:0001184 | WBPaper0037950_ hypodermis_embryos_enriched_, WBbt_0005190_361 | −0.12±0.09 | 4.11          | 1.04          |
| pck-1_22220-pck-1_33 | R11 C12        | Aging GO:0007568 | Sclerotic WBPhenotype:0000646 | WBPaper0037950_ hypodermis_embryos_enriched_, WBbt_0005190_361 | −0.11±0   | 1.85          | 1.69          |
| iff-2_18754-rpl-25.1 | R12 C37        | Amide biosynthetic process GO:0043604 | Hermaphrodite fertility reduced WBPhenotype:0001259 | WBPaper0037950_ hypodermis_embryos_enriched_, WBbt_0005190_361 | −0.11±0.03 | 0.65          | 1.39          |

Table 3. Most strongly affected cliques by *unc-45* RNAi and their characteristics. Clique positions (letter = row, number = position from left to right) correspond to the clique map shown in Fig. 2b.
| Series     | Sample          | Description                | Replicate |
|------------|-----------------|----------------------------|-----------|
| GSE6547    | GSM146422       | N2 worms at L1 stage       | 1         |
|            | GSM146423       | N2 worms at L1 stage       | 2         |
|            | GSM147330       | N2 worms at L1 stage       | 3         |
|            | GSM147334       | N2 worms at L4 stage       | 1         |
|            | GSM147335       | N2 worms at L4 stage       | 2         |
|            | GSM147336       | N2 worms at L4 stage       | 3         |
|            | GSM147340       | N2 worms at embryonic stage| 1         |
|            | GSM147341       | N2 worms at embryonic stage| 2         |
|            | GSM147342       | N2 worms at embryonic stage| 3         |
| GSE21784   | GSM542652       | L4 larvae                  | 1         |
|            | GSM542653       | L4 larvae                  | 2         |
|            | GSM542654       | L4 larvae                  | 3         |
|            | GSM542655       | Day 6 adult                | 1         |
|            | GSM542656       | Day 6 adult                | 2         |
|            | GSM542657       | Day 6 adult                | 3         |
|            | GSM542658       | Day 15 adult               | 1         |
|            | GSM542659       | Day 15 adult               | 2         |
|            | GSM542660       | Day 15 adult               | 3         |
| GSE46288   | GSM1128166      | L3                         | 1         |
|            | GSM1128167      | L3                         | 2         |
|            | GSM1128168      | L3                         | 3         |
|            | GSM1128169      | L3-lethargus               | 1         |
|            | GSM1128170      | L3-lethargus               | 2         |
|            | GSM1128171      | L3-lethargus               | 3         |
| GSE46289   | GSM1128172      | L4                         | 1         |
|            | GSM1128173      | L4                         | 2         |
|            | GSM1128174      | L4                         | 3         |
|            | GSM1128175      | L4                         | 4         |
|            | GSM1128176      | L4                         | 5         |
|            | GSM1128177      | L4-lethargus               | 1         |
|            | GSM1128178      | L4-lethargus               | 2         |
|            | GSM1128179      | L4-lethargus               | 3         |
|            | GSM1128180      | L4-lethargus               | 4         |
|            | GSM1128181      | L4-lethargus               | 5         |
|            | GSM1128182      | Adult                      | 1         |
|            | GSM1128183      | Adult                      | 2         |
|            | GSM1128184      | Adult                      | 3         |
|            | GSM1128185      | Adult                      | 4         |
|            | GSM1128186      | Adult                      | 5         |

Table 4. Experiments used for the analysis of the developmental time line of *C. elegans*, obtained from the GEO expression data repository.
Figure 3. Expression in developmental stages is altered in similarity to the RNAi-induced arrest. (a) L3 comparison to young adult. (b) L4 comparison to young adult and (c) L4 lethargus to young adult. Other stepwise comparisons, like that of embryo to L1 or that of day6 nematode to day 16 adult nematode are shown in the Supplemental Figures section as Supplemental Fig. 3, 4, 5 and 6. (d) Clique responses throughout development shown for selected cliques with significant change pattern. Cliques colored in red are induced compared to the earlier developmental stage, while cliques colored in blue are repressed. The linear regression function was generated with Microsoft Excel without weighting, a square value of 1 would indicate a perfect correlation between the cliques.
| Up1  | Up2  | Down1      | Down2      | Down3      | Down4      |
|------|------|------------|------------|------------|------------|
| C10C5.2-Y58A7A.3 | col-138-col-49 | C46C2.5_15926-W03F11.1 | C17E7.4-T06D4.1 | vit-2-vit-4_22519 | C17H12.6-swt-6 |
| K08D10.9-F46A8.1 | R12E2.14_75-R12E2.15 | ZC373.2-Y62H9A.6_1596 | fbx-28-sdz-28 | C10A4.6-C01A2.6 | F55G11.8-dod-17 |
| hsp-16.2-F44E5.4_19238 | mh9.22518-F33D4.6_14044 | C10C5.3-C10C5.5 | sea-1-R04D3.4 | fbx-28-sdz-28 | R10H10.3-T21H3.1 |
| lys-3-tsp-1 | col-117-col-167_1015 | 171971_x-at-D1054.11_184 | ZK1053.4-C08F1.6 | C17E7.4-T06D4.1 |
| hpo-6-C49C3.9 | col-146-col-133 | C46C2.5_15925-F17B9.2 | F11A3.2-F47G3.3 | R07E4.5-skp-1_1608 |
| C32F10.4-D1086.2 | bus-8_3160-K04H4.2_2324 | C03B1.14-F46C3.2 | K09D9.12-T10C6.10 | R10H10.3-T21H3.1 |
| F13E6.1_2090-C35C3.3_1843 | sqt-2-dpy-9 | col-178-col-19 | K05C4.4-fbx-46 | sre-33-ZK1025.1_8337 |
| Y6G8.2_5650-F17B5.1 | abu-7-abu-8_22491 | T24D1.3-egg-1 | fbx-31-fbx-119 |
| fbx-35-Y39A3A.3 | agmo-1_5527-F53B1.4 | Y116A8C.19-F38C2.7 |
| C17H1.6-C17H1.13 | dos-2-grd-2 | npp-8_4368-E01B7.1_7741 |
| ceg-1_12351-ZC410.5 | R12A1.3-M195.2 | szd-10-fbx-62 |
| C17H12.6-swt-6 | psn-54-abu-9 | fbx-13-fbx-24 |
| F58F9.3_11574-R07B7.6 | col-128-cdh-10_9234 | skr-16-F37B4.10 |
| T12G3.1_17-T12G3.1_18846 | C38C6.3-acdh-6 | dsh-1_3575-C40A11.4 |
| W01F3.2_1554-Y106G6D.8 | C36C5.12-F57G8.7 | dos-2-grd-2 |

**Table 5.** HSP-90-responsive cliques with highest enrichment of *daf-16* supported (red) and suppressed (blue) genes. Sorted according to the nomenclature established in Eckl et al., 2017, which grouped the hsp-90 responsive genes in two major groups for upregulation and four groups for downregulation based on co-expression network analysis. Cliques enriched within these groups are listed under the group names (Up1, Up2, Down1, Down2, Down3 and Down4). The groups are labeled in bold, if enrichment reaches the significance level of 1e-5 and they are colored, if the same group is found within the top *daf-16* regulated groups. Coloring is red, if it is among upregulated DAF-16 targets and blue if, it is among down regulated DAF-16 targets.
Figure 4. Correlation between *daf-16* target genes and identified cliques for those genes most clearly enriched in *daf-16* targets. (a) Enriched target cliques of DAF-16. The plot shows how many genes per clique are derived from the indicated range of the DAF-16 ranking. Cliques with high percentage values on the left side reflect cliques that are considered to be DAF-16 activatable while cliques with a low percentage up to the bottom to the DAF-16 ranking are considered repressed. (b) Most strongly enriched cliques in the Top750 and Bottom750 of the ranked *daf-16* target list.
Data availability
All data will be made fully available without restriction at http://www.richterlab.de/DataSets and on the GEO repository. Tables containing GO terms, PEA and TEA enrichment results for all cliques can be obtained from the authors.

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**Author contributions**

L.S. and K.R. designed the experiments. L.S. performed the experiments. L.S. and K.R. analyzed the data and wrote the manuscript.

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