Supplementary material from "Uncovering the Mechanisms of *C. elegans* Ageing from Global Quantification of Underlying Landscape"

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1 Procedures of Network Construction

The network method is widely used to simulate the system-level features of simplified system. For such a complicated process like aging, innumerable elements and relations are involved. Therefore, a core gene regulatory network with the most important genes and interactions is constructed to explore the basic features of the *C. elegans* aging process.

We started the network construction from some widely studied pathways such as IIS, TOR and AMPK, for the biological functions. How these pathways influence *C. elegans* lifespan have already been discussed, and regulatory information among the involved genes have also been studied. The evidences are collected from the literatures which identified the regulations with low through-put biological experiments. After that, some other genes that either connect different pathways or form feedback loops with the existing pathways were added to the network. These genes may not have very important functions themselves, but can influence the system behavior by communicating with core genes. Finally, we simplified the network by removing the genes that do not regulate others and combining multi-step reactions into a single regulatory interaction. The complexes integrated by different proteins and microRNAs are also treated as one gene node for consistent presentation. This simplification can reduce computational complexity when quantifying the global landscape topography and give relatively simpler and clearer results. Through these processes, our *C. elegans* aging network with 11 genes and 27 regulations was constructed(Fig.1).

2 Parameters of Dynamical Equations

The choice of parameters may significantly impact the system behaviors. However, these is no widely accepted suggestion on how to set the parameters. In this work, we set the parameters in Eq.1 according to the following criteria:

1. We choose the parameters by the experiences from previous works on gene regulations. We set the Hill coefficient $n = 3$, and the self-degradation

rate $\mu_k = 1$ in our model[1, 2]. Moreover, the barrier heights and noises are the key factors determining the state transitions of ageing. However, up to now, there is no direct experiments measuring the noise level and the barrier heights for ageing yet. So we chose the noise level according to some previous works on gene regulatory networks[3, 4]. The barrier height can be obtained from the probability or histograms of the gene expressions. In our bistable landscape shown in Fig.2, the barrier height (BH) from ageing to rejuvenation attractor is 2.2701, and the BH from rejuvenation to ageing attractor is 1.2653. The Gaussian noise determined by the diffusion coefficient D is set to 0.01.

2. We set the parameters aim to reduce the complexity and make the result clear. For example, we set the parameter w_k equal for each target gene, and if there are totally N regulations targeting to gene k, we assumed all $w_k = 1/N$, this assumption can keep all the gene expression values between 0 and 1. We set parameter $s = 0.5$ for all regulations in Fig.1, which makes all the regulations with the same regulation strength for there is no direct approach to get the real value. The difference in regulations then comes from the expression levels rather than the numerical values of the regulation strengths (the regulation is determined by both the regulation strength and the expression levels of the regulatory genes).

3 Entropy Production Rate and Flux Integrals

The change of entropy in time of the non-equilibrium system can be divided into two terms[5, 6] : $\dot{S} = \dot{S}_t - \dot{S}_e$, where the $\dot{S}_t$ represents the entropy production rate(EPR), $\dot{S}_t = \int d\mathbf{x}(\mathbf{J} \cdot (D\mathbf{D})^{-1} \cdot \mathbf{J})/P$, and the $\dot{S}_e$ represents the heat dissipation rate to the environments, $\dot{S}_e = \int d\mathbf{x}(\mathbf{J} \cdot (D\mathbf{D})^{-1} \cdot \mathbf{F}')$, the effective force $\mathbf{F}' = \mathbf{F} - D\nabla \cdot \mathbf{D}$.

The EPR indicates the total entropy change of both the system and the environment around, which is non-negative for the thermodynamic second law. The entropy change of the non-equilibrium system itself can be positive or negative, due to the entropy flow from or into the environments.

The EPR is highly correlated with the probability flux, we define the flux integral as : $Flux_{int} = \int |\mathbf{J}| d\mathbf{x} / \int d\mathbf{x}$. Here the integral $\int$ is along a closed loop. For oscillation, a natural closed loop can be chosen as the oscillation path. Larger flux leads to faster speed in the oscillation case and more dissipation.

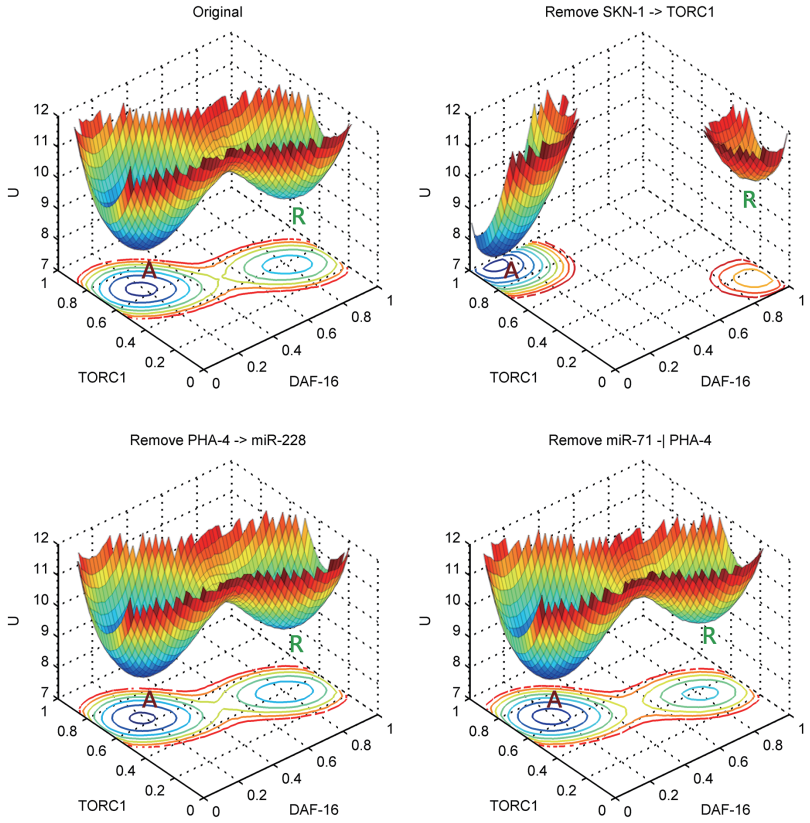

Fig S1: Genes perform longevity-promoting and lifespan-limiting functions are colored as green and red in Fig 1. There are three exceptional regulations, SKN-1 $\rightarrow$ TORC1, PHA-4 $\rightarrow$ miR-228 and miR-71 $\dashv$ PHA-4, that either activating other genes in their own group or repressing genes in the other group in our network. The four landscape subgraphs according to the original network, and after separately remove the three exceptional regulations. The ageing and rejuvenation attractors are labeled as A and R in each subgraph.

Table S1: Evidences for the regulations in the worm ageing network.

ID	Regulation	Reference
1	DAF-16 ⊣ TORC1	[7]
2	DAF-16 ⊣ DAF-2	[8]
3	DAF-16 → AAKG-4	[9, 10]
4	TORC1 ⊣ DAF-16	[11]
5	TORC1 ⊣ SKN-1	[11]
6	TORC1 → RSKS-1	[12]
7	SKN-1 → TORC1	[11]
8	SKN-1 ⊣ DAF-2	[13]
9	SKN-1 → miR-71	[14]
10	SKN-1 ⊣ miR-228	[14]
11	DAF-2 ⊣ DAF-16	[15]
12	DAF-2 ⊣ SKN-1	[16]
13	DAF-2 ⊣ AAK-2	[17]
14	AAK-2 → DAF-16	[18, 10]
15	AAK-2 ⊣ TORC1	[19]
16	AAK-2 → SKN-1	[20]
17	AAK-2 ⊣ HIF-1	[21]
18	AAKG-4 → AAK-2	[10]
19	RSKS-1 ⊣ AAK-2	[10]
20	RSKS-1 → HIF-1	[22]
21	RSKS-1 ⊣ PHA-4	[23]
22	HIF-1 ⊣ DAF-16	[24]
23	PHA-4 → miR-228	[14]
24	miR-71 ⊣ DAF-2	[25]
25	miR-71 ⊣ PHA-4	[14]
26	miR-228 ⊣ SKN-1	[14]
27	miR-228 ⊣ PHA-4	[14]

Table S2: Gene expressions of the ageing and rejuvenation states in Fig.2.

System state		Aging	Rejuvenation
Longevity promoting genes	DAF-16	0.149	0.865
	SKN-1	0.168	0.791
	AAK-2	0.128	0.869
	AAKG-4	0.026	0.838
	PHA-4	0.502	0.565
	miR-71	0.037	0.798
Lifespan limiting genes	TORC1	0.665	0.373
	DAF-2	0.979	0.187
	RSKS-1	0.702	0.294
	HIF-1	0.859	0.164
	miR-228	0.733	0.396

Table S3: Regulation strengths of the oscillation dynamics.

ID	Regulation	Strength
1	DAF-16 ⊣ TORC1	0.763926
2	DAF-16 ⊣ DAF-2	0.555771
3	DAF-16 → AAKG-4	0.253207
4	TORC1 ⊣ DAF-16	0.317654
5	TORC1 ⊣ SKN-1	0.868
6	TORC1 → RSKS-1	0.276751
7	SKN-1 → TORC1	0.875509
8	SKN-1 ⊣ DAF-2	0.849966
9	SKN-1 → miR-71	0.847142
10	SKN-1 ⊣ miR-228	0.356759
11	DAF-2 ⊣ DAF-16	0.429313
12	DAF-2 ⊣ SKN-1	0.408048
13	DAF-2 ⊣ AAK-2	0.878923
14	AAK-2 → DAF-16	0.124333
15	AAK-2 ⊣ TORC1	0.782731
16	AAK-2 → SKN-1	0.364681
17	AAK-2 ⊣ HIF-1	0.762594
18	AAKG-4 → AAK-2	0.079872
19	RSKS-1 ⊣ AAK-2	0.833707
20	RSKS-1 → HIF-1	0.059585
21	RSKS-1 ⊣ PHA-4	0.27733
22	HIF-1 ⊣ DAF-16	0.651354
23	PHA-4 → miR-228	0.474354
24	miR-71 ⊣ DAF-2	0.707569
25	miR-71 ⊣ PHA-4	0.326844
26	miR-228 ⊣ SKN-1	0.722264
27	miR-228 ⊣ PHA-4	0.763233

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