Direct inhibition of the longevity promoting factor SKN-1 by Insulin-like signaling in C elegans

Tullet, J. M. A., Hertweck, M., Hyung An, J., Baker, J., Hwang, J. Y., Liu, S., Blackwell, T. K. (2008). Direct inhibition of the longevity promoting factor SKN-1 by Insulin-like signaling in *C. elegans*. *Cell*, *132*(6), 1025–1038. http://doi.org/10.1016/j.cell.2008.01.030

The insulin/insulin-like signaling (IIS) pathway is integral to metabolism in many organisms. The inhibition or reduction in IIS in C elegans increases stress resistance, which has been shown to require the IIS-inhibited transcription factor DAF-16. The transcription factor SKN-1 has been shown to be involved in the phase 2 detoxification responses against oxidative stress. Studies have shown that the IIS pathway directly inhibits DAF-16 pathways leading to stress resistance and longevity. The scientists of this experiment reasoned that if IIS inhibits stress response of DAF-16, it might also oppose SKN-1.

To determine whether IIS inhibits SKN-1 similar to DAF-16, RNA interference was used to reduce certain gene expression. It was proposed that if IIS inhibits SKN-1, reduced DAF-2 signaling (first receptor of IIS) would allow SKN-1 accumulation in intestinal nuclei. I To investigate this, DAF-2 activity was reduced using RNAi and SKN-1 was shown to constitutively accumulate in the intestinal nuclei. IIS inhibits DAF-16 through phosphorylation, therefore the experiment also investigated whether this directly inhibits SKN-1 similarly. RNAi knockdown of the phosphorylating akt-1, akt-2 and sgk-1 showed significant increase in the presence of SKN-1::GFP which further supported the model that IIS is able to directly inhibit SKN-1.

Finally the experiment also investigated the proposition that if IIS directly inhibits SKN-1, it should also reduce the expression of the target genes. RNAi of the phosphorylating factors mentioned earlier also showed an increase in gcs-1, however RNAi of DAF-2 did not induce gcs-1, showing that akt and sgk-1 might have additional input. Quantitative PCR was used to see how a DAF-2 mutation which would inhibit IIS, affects the levels of mRNA from a set of known SKN-1 target genes involved in phase 2 detoxification. This showed up-regulation of some target genes, whereas some remained unaffected. These results indicated that DAF-2 pathway reduced signaling (IIS reduction) affects SKN-1 in a way that overlaps with DAF-16. Therefore, the findings concluded that the transcription network regulated by SKN-1 that promotes longevity is a direct target of IIS.