Synergistic effects of feeding and eyestalk ablation on ovarian maturation of the black tiger shrimp

Kanchana Sittikankaew[1], Wirulda Pootakham[2], Chutima Sonthirod[2], Duangjai Sangsrakru[2], Thippawan Yoocha[2], Jutatip Khudet[2], Intawat Nookaew[3], Umaporn Uawisetwathana[2], Wanilada Rungrassamee[2], Nitsara Karoonuthaisiri[1]

[1]National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency, Pathum Thani 12120, Thailand

[2]National Omics Center, National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency, Pathum Thani, Thailand

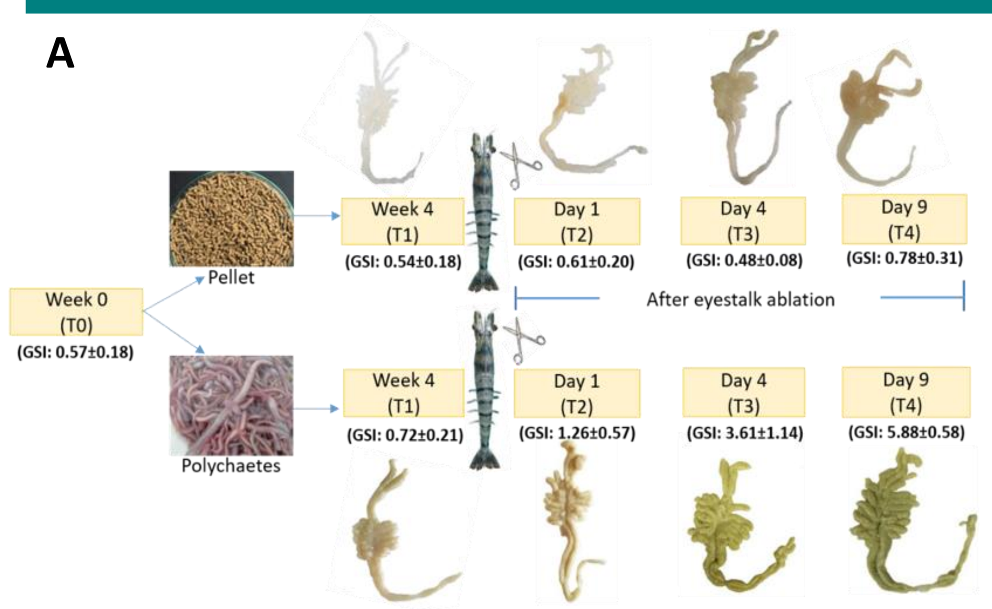
[3]College of Medicine, Department Biomedical Informatics, University of Arkansas for Medical Sciences, Little Rock, AR 72205, USA

Kanchana.sit@biotec.or.th

Abstract

Unilateral ablation of eyestalk in female black tiger shrimp *Penaeus monodon* is known to induce ovarian maturation. However, without feeding the female broodstock with live feeds, the inductive effect cannot be realized. Thus, the synergistic effects of feeding with live feeds and the ablation must be elucidated. This study was conducted when the high-quality genome assembly was not available, thus it constructed the first high-quality ovarian reference transcriptome in *P. monodon* using short-read Illumina RNA sequencing and long-read Pacific Biosciences (PacBio) isoform sequencing (Iso-seq). This transcriptome assembly allowed us to dissect the effects of feeds and eyestalk ablation and reveal their synergistic effects at the transcriptomic level. We found the regulation of important genes involved in fatty acid regulation, energy production, and hormone-mediated oocyte maturation pathways. These findings shed the light on molecular mechanisms and key molecular pathways which may lead to means to induce ovarian maturation without having to perform eyestalk ablation in the future.

Experimental design and sample collection



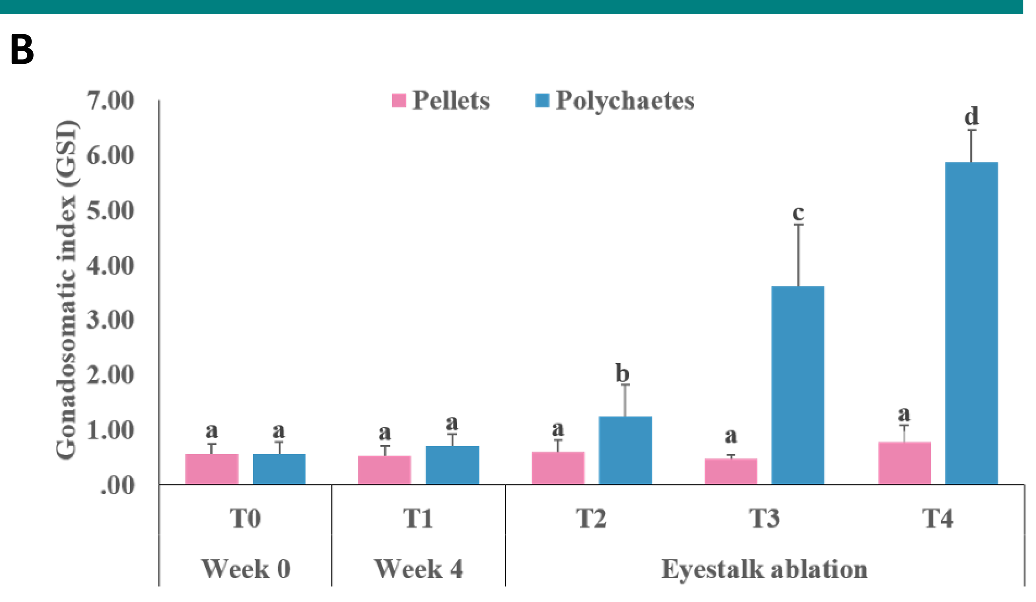


Fig 1. Ovarian maturation after unilateral eyestalk ablation in the shrimp fed with pellet or polychaetes. (A) Representatives of ovaries from different time points. (B) Gonadosomatic Index (GSI) of the shrimp at different time points. Error bars represent standard derivation. Different letters indicate significant differences among time points of the same feed group (p<0.05, ANOVA *Duncan test*). Asterisks indicate significant differences between the two feed groups at the same time point (p<0.05, *t-test*).

RNA extraction → Library preparation → Sequencing → Data analysis

RNA extraction

- Iso-seq: Pooled T0, T1, T2, T3, T4 (N=1/each group)
- RNA-seq: Individual T0, T1, T2, T3, T4 (N=2/each group)

Library preparation

- SMARTer PCR cDNA Synthesis Kit (Clontech, Mountain View, USA)
- TruSeq stranded Library Preparation kit (Illumina, San Diego, USA)

Sequencing

- PacBio RS II instrument
- Illumina NovaSeq™ 6000 instrument (150 bp pair end read)

Data analysis

- Transcriptome assembly and annotation
- Mapping and differential gene expression analysis

Result

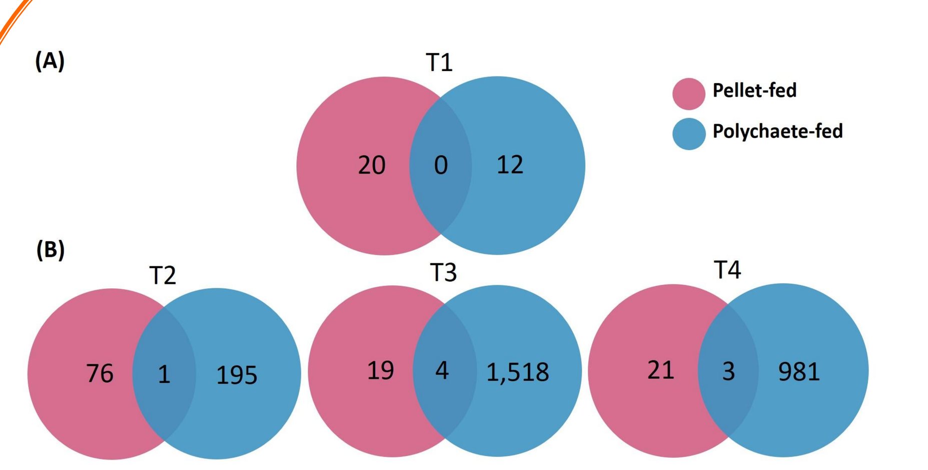


Fig 2. Venn diagram of DEGs of the pellet-fed shrimp and the polychaete-fed shrimp (A) after the 4-week feeding trial (T1) and (B) after eyestalk ablation at Day 1, 4, and 9 (T2, T3, and T4, respectively).

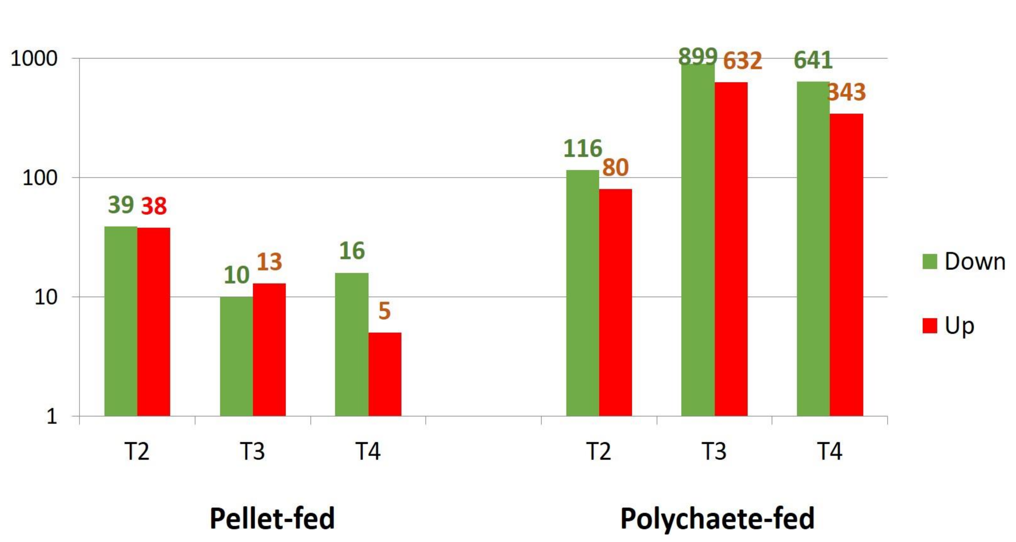


Fig 3. Numbers of DEGs after eyestalk ablation of the pellet-fed shrimp and the polychaete-fed shrimp at Day 1, 4, and 9 (T2, T3, and T4, respectively). Red and green bars indicate up- and downregulated DEGs. A base-10 log scale is used for the Y axis.

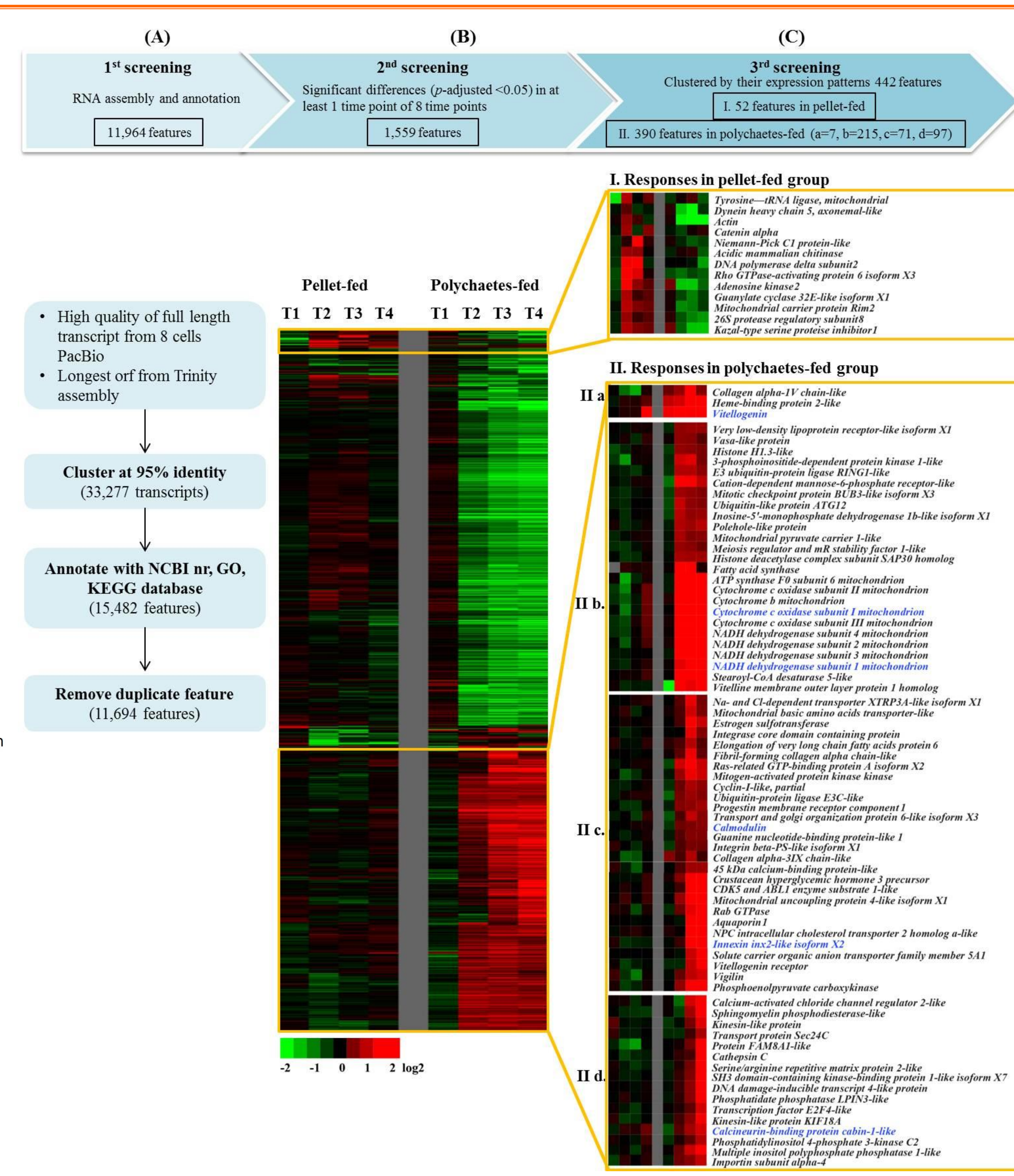


Fig 4. Gene expression analysis by RNA sequencing. Transcript levels in ovaries after a 4-week feeding trial (T1) were compared to before the trial (T0). Transcript levels in ovaries after eyestalk ablation at Day 1, 4, and 9 (T2, T3, and T4, respectively) were compared to those from non-ablated broodstock (T1). (A) A total of non-redundant and annotated 11,964 features were obtained from RNA sequencing. (B) Hierarchical clustering analysis of the 1,559 features with significant differences (p-adjusted <0.05) in at least 1 time point of 8 time points. (C) Genes that exhibited changes in there expression level>1.5 folds (442 features) were clustered into Cluster I-II exhibiting various differentially expressed patterns as effects of diets and eyestalk ablation: Upregulation in pellet-fed group (I), Upregulation in polychaete-fed group (II) with cluster IIa contains genes with upregulation before and after the ablation, cluster IIb contains genes with upregulation after the ablation until Day 9, cluster IIc contains genes with upregulation at mid time point after the ablation, and cluster IId contains genes with upregulation at later time point after the ablation.

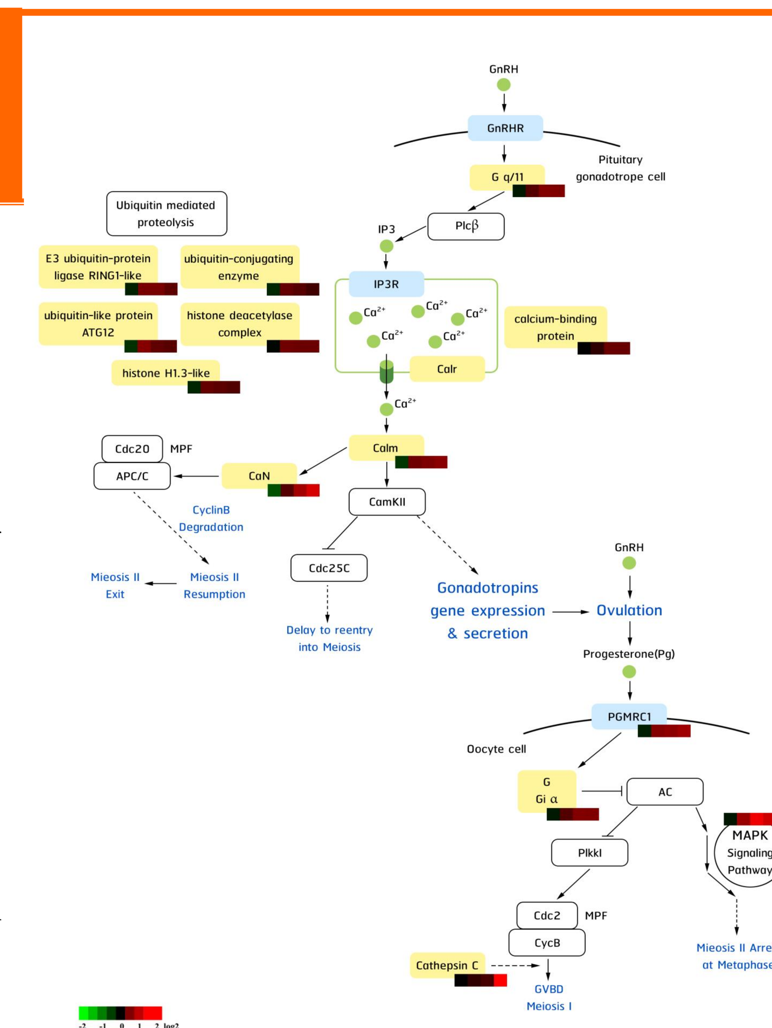


Figure 5. Differential expressed genes in the hormone-mediated ovarian maturation pathways in the polychaete-fed group. Light blue box, yellow box and white box represent receptor genes, genes were found in this study, and genes known to be in this pathway, respectively. The heatmap bars under each gene in the pathway reflect expression change from RNA-seq result after a 4-week feeding trial (T1) compared to before the trial (T0), after eyestalk ablation at Day 1, 4, and 9 (T2, T3, and T4, respectively) compared to those from non-ablated broodstock (T1).

Conclusion

- **RNA-seq results strongly suggest that there are synergistic effects between the polychaete feeding and the eyestalk ablation in the process of ovarian maturation in black tiger shrimp**
- **The eyestalk ablation is still necessary to perhaps manipulate the female endocrine of the black tiger shrimp, but this technique was synergist with nutrition diet in polycheates**
- **The synergy seems to be the induction of important groups of genes, namely energy production genes, endocrinological genes, fatty acid regulatory genes, and oogenesis genes**
- **These findings shed to light on molecular mechanisms and key molecular pathways that lead to successful ovarian maturation**

Reference:

Sittikankaew, K., Pootakham, W., Sonthirod, C., Sangsrakru, D., Yoocha, T., Khudet, J., Nookaew, I., Uawisetwathana, U., Rungrassamee, W., Karoonuthaisiri, N., Transcriptome analyses reveal the synergistic effects of feeding and eyestalk ablation on ovarian maturation in the black tiger shrimp (*Penaeus monodon*), Scientific report., ttps://doi.org/10.1038/s41598-020-60192-2

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