Academicia Globe: Inderscience Research

ISSN: 2776-1010 Volume 3, Issue 2, Feb, 2022

PLECKSTRIN HOMOLOGY AND GREEN FLUORESCENT FUSION PROTEIN IN STARFISH BY USING BIOINFORMATICS

Rana Hussein Naser,
Department of Science, College of Basic Education, University of Diyala, Iraq

Israa Tareq Aakool,
Department of Science, College of Basic Education, University of Diyala, Iraq

Arwa Alaa Hussein
Department of Science, College of Basic Education, University of Diyala, Iraq

Abstract
Different signaling and metabolic processes, including those that occur during fertilization, are tightly controlled by PLC isozymes. In this research, Bioinformatic databases will be utilized to fuse PLC's PH domain with GFP, which will then be used to study the starfish Patiria miniata's starfish chromosomes.

Keywords: Infertility, Phospholipase C, GFP, Patiria miniata

Introduction

In order to stimulate embryogenesis, cytoplasmic free Ca2+ is detected in the eggs. increase at fertilization [1] [2]. Anti-vertebrate protein inhibitors have been used in research in starfish eggs have been offered that this C2+ rise necessitates SFK (Src family kinase) in the egg that either activates PLC-gamma directly or indirectly, resulting in IP3 production, it causes C2+ to be released from the endoplasmic reticulum of the egg (ER). Asterina miniata PLC-gamma was obtained from oocyte cDNA to study the endogenous measures in starfish eggs necessary for C2+ release during fertilization in greater detail. AmPLC gamma is a cDNA that encodes a protein that is 49 percent identical PLC-gamma in mammalians. Recombinant Src homology 2 (SH2) domains in AmPLC-gamma interacted with a 58-kDa Src family kinase in a fertilization-responsive way [3] [4]. PLC from a sea urchin egg immunoprecipitates the PLC-gamma was shown to be phosphorylated in response to fertilization when it was tested with an antibody specific against AmPLC-gamma. Adding starfish eggs to the mix with AmPLC tandem gamma's SH2 domains (which block activation of PLC gamma) prevented release of Ca2+ at fertilization. These findings show that an endogenous starfish egg PLC-gamma interacts with an egg SFK and, via a PLC-gamma SH2-mediated mechanism, mediates Ca2+ release during fertilization [5] [6].Calcium signaling levels are maintained by the isoform PLCγ, which assist to open a channel that allows for Ca2+ infusion over the plasma membrane and out of the endoplasmic reticulum, respectively [7]. PLC1 and PLC2 are two isoforms of the PLC class, growth factor stimulation of receptor and non-receptor (cytosolic) protein tyrosine kinase activation by polypeptide growth factor resulting in an increase in the activity of phospholipase, which can lead to angiogenesis, cell motility,

ISSN: 2776-1010 Volume 3, Issue 2, Feb, 2022

ventricular contractility, among other things [8] [9]. GFP (Green fluorescent protein) is a widely recognized and transiently expressed fluorescent tag that can play a vital function in the localization of PLCγ. GFP will be fused to PLC's PH-domain. In this work, and the PH-GFP fusion protein will be utilized to investigate localisation in the starfish Patiria miniata [10]. Additional PLC family members have been demonstrated to influence Ca2+ signaling via previously undiscovered mechanisms, which suggests that this fusion protein may also Other non-membrane cytosolic proteins interact with and localize to compartments when exposed to sperm-egg interaction. Because the PH domain aids in marked protein-protein, protein-lipid interactions, and membrane binding it is used in this work for the development of fusion proteins that can bind to membranes [11] [12].

Materials and Methods

1-NCBI The NCBI houses a series of (computer files full of information) clearly connected with or related to (science that uses living things to improve the Earth) and natural communitydicine and is an important useful thing supply for bioinformatics tools and services [13]. Major computer files full of information include GenBank for DNA sequences and PubMed, a related to a list of references, computer file full of information for the study of how life and medicine work together. Other computer files full of information include the NCBI Epigenomics. All these computer files full of information are available online through the Entrez search engine [14].

2- Bioinformatics To construct a PH-GFP fusion protein, PLC PH domain of starfish PLC was amplified using bioinformatics to construct primers containing BsrG1 restriction sites the PJV53 – PAGFP plasmid [15] [16]. The NCBI database was used to retrieve the cDNA sequence in its entirety and the PH domain of AmPLCγ. PH domain was amplified by using NCBI's Primer-Blast to build the forward and reverse primers [17] [18] [19].

Result and Discussion

Asterina miniata phospholipase C-gamma mRNA, complete cds

GenBank: AY486068.1

FASTA Graphics

Go to:

```
LOCUS       AY486068                3816 bp    mRNA    linear   INV 14-APR-2004
DEFINITION  Asterina miniata phospholipase C-gamma mRNA, complete cds.
ACCESSION   AY486068
VERSION     AY486068.1
KEYWORDS    .
SOURCE      Patiria miniata (bat star)
  ORGANISM  Patiria miniata
            Eukaryota; Metazoa; Echinodermata; Eleutherozoa; Asterozoa;
            Asteroidea; Valvatacea; Valvatida; Asterinidae; Patiria.
REFERENCE   1  (bases 1 to 3816)
  AUTHORS   Runft,L.L., Carroll,D.J., Gillett,J., Giusti,A.F., O'Neill,F.J. and
            Foltz,K.R.
  TITLE     Identification of a starfish egg PLC-gamma that regulates Ca2+
            release at fertilization
  JOURNAL   Dev. Biol. 269 (1), 220-236 (2004)
   PUBMED   15081369
REFERENCE   2  (bases 1 to 3816)
  AUTHORS   Gillett,J., Carroll,D.J., Runft,L.L., O'Neill,F.J., Giusti,A.F.,
```

Figure 1. Initial results page of the nucleotide search for Asterina miniata phospholipase C-gamma mRNA. https://www.ncbi.nlm.nih.gov/nuccore/40365362?log$=activity

ISSN: 2776-1010 Volume 3, Issue 2, Feb, 2022

phospholipase C-gamma [Patiria miniata]

GenBank: AAR85355.1

Identical Proteins FASTA Graphics

Go to:

```
LOCUS       AAR85355                1261 aa            linear   INV 14-APR-2004
DEFINITION  phospholipase C-gamma [Patiria miniata].
ACCESSION   AAR85355
VERSION     AAR85355.1
DBSOURCE    accession AY486068.1
KEYWORDS    .
SOURCE      Patiria miniata (bat star)
  ORGANISM  Patiria miniata
            Eukaryota; Metazoa; Echinodermata; Eleutherozoa; Asterozoa;
            Asteroidea; Valvatacea; Valvatida; Asterinidae; Patiria.
REFERENCE   1  (residues 1 to 1261)
  AUTHORS   Runft,L.L., Carroll,D.J., Gillett,J., Giusti,A.F., O'Neill,F.J. and
            Foltz,K.R.
  TITLE     Identification of a starfish egg PLC-gamma that regulates Ca2+
            release at fertilization
  JOURNAL   Dev. Biol. 269 (1), 220-236 (2004)
   PUBMED   15081369
REFERENCE   2  (residues 1 to 1261)
  AUTHORS   Gillett,J., Carroll,D.J., Runft,L.L., O'Neill,F.J., Giusti,A.F.,
            Jaffe,L.A. and Foltz,K.R.
  TITLE     Direct Submission
  JOURNAL   Submitted (24-NOV-2003) Biological Sciences, Florida Tech, 150 West
            University Blvd., Melbourne, FL 32901, USA
ORIGIN
        1 matnslykkk ltpqevasvt kmlkmgtvlt rfygkrrper rsfeicmetr qilwrrqtgr
       61 tdgavkirei keirpgknsr dferwpdeak kydtslclvi cygaefrlks lsvvagnade
      121 rhkwivglnw lvedhkissy psrlewwlrr efyamgktkn dtvslrdmks fmpyvnlkmn
      181 tkdlkeyfne vdrwnkqeig fdgfvqlyhn lifqrevadr fkeyidernl vtvngmirfl
      241 aqeqkdttan npiavkamme sfltdlgrpc qesdpkftvp efllylfspd neiwdkkfde
```

Figure 2. The Asterina miniata src cDNA was translated to Patiria miniata src protein. Amino acid sequence of the Patiria miniata Src family kinase protein, with the ph domain highlighted in brown from range(23-141).
https://www.ncbi.nlm.nih.gov/protein/40365363

Academicia Globe:
Inderscience Research

ISSN: 2776-1010 Volume 3, Issue 2, Feb, 2022

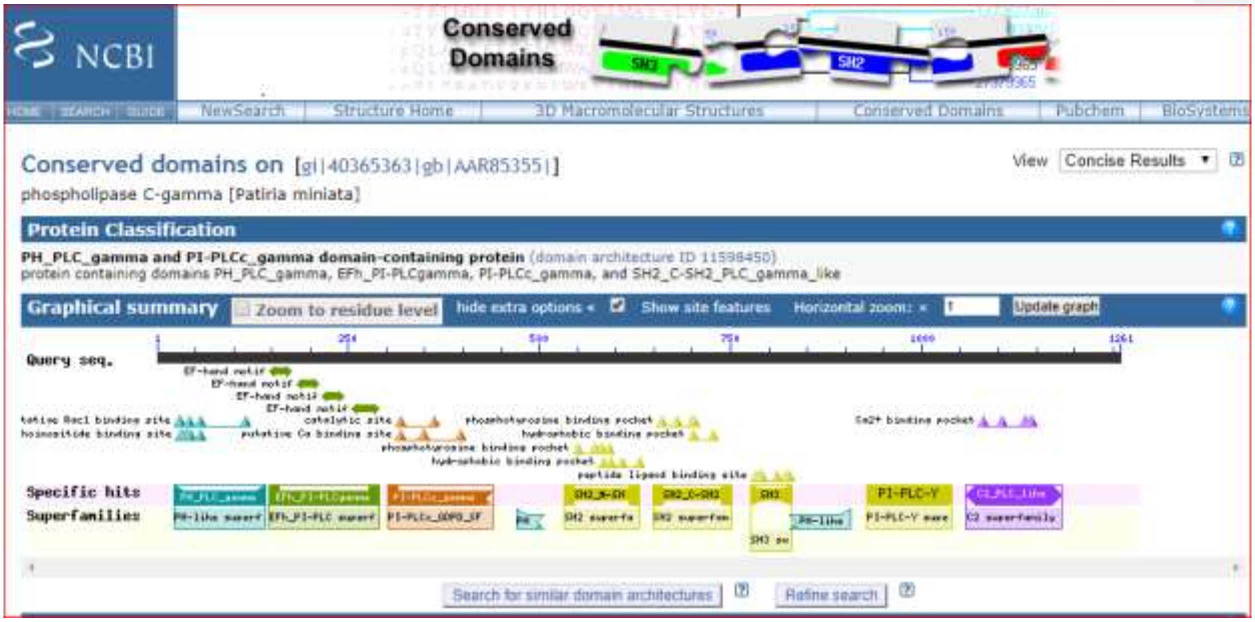
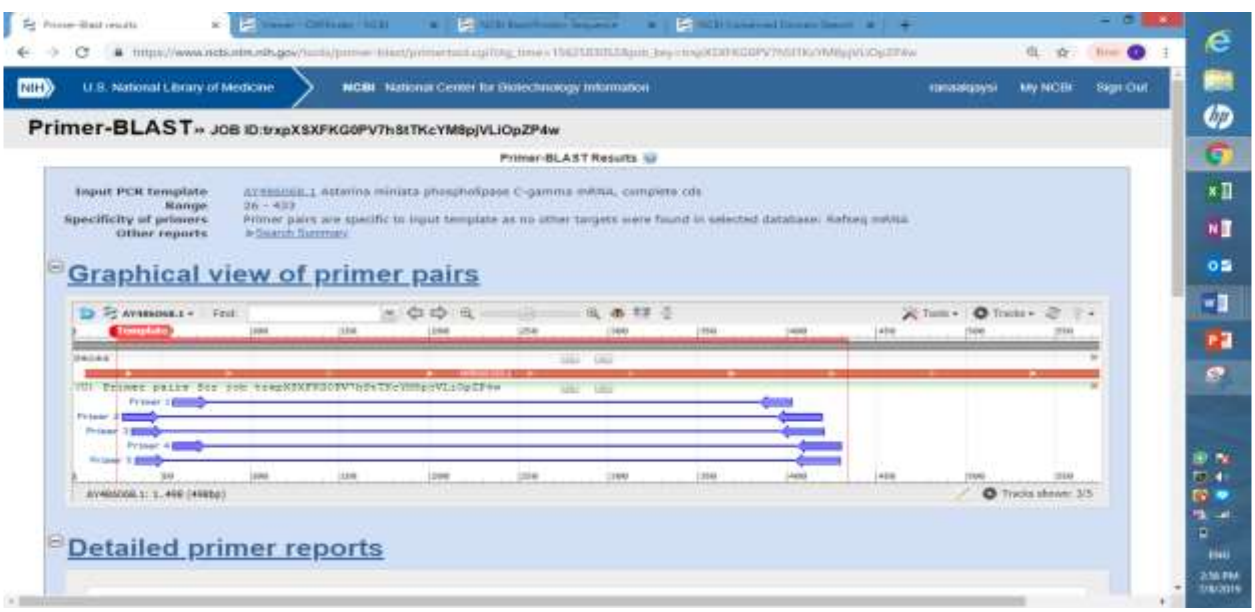


Figure 3. Conserved domain results for patiria minita . Conserved domain compares this protein sequence to the sequence for the same protein in other animals and identifies regions of high similarity (conserved regions). This shows that the ph domain is highly conserved in this protein. https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi?INPUT_TYPE=live&SEQUENCE=AAR85355.1



ISSN: 2776-1010 Volume 3, Issue 2, Feb, 2022

Primer pair 2

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	AAGAAGAAGCTGACGCCCCA	Plus	20	29	48	61.77	55.00	4.00	2.00
Reverse primer	AGATTTTATGGTCTTCCACTAGCCA	Minus	25	419	395	59.81	40.00	4.00	2.00
Product length	391								

Primer pair 3

https://www.ncbi.nlm.nih.gov/tools/primer-blast/primertool.cgi?ctg_time=1562583052&job_key=trxpXSXFKG0PV7hStTKcYM8pjVLiOpZP4w
Figurre 4: primer-blast from NCBI are used to design forward primer and reverse primer , Next these primrs will cut out by using restriction enzyme BrsGI

```
ORIGIN
        1 cttcagaatg gccaccaaca gcctctacaa gaagaagctg acgccccagg aggtggccag
       61 cgtcaccaag atgctgaaaa tgggcaccgt cctgacgcgc ttctacggca aacgacgacc
      121 ggaaaggagg tcgttcgaaa tctgcatgga gacgcggcag atactgtgga ggcgacagac
      181 tgggcggaca gacggagcag ttaaaattcg tgagataaaa gagattcgtc ccggtaagaa
      241 ctcacgagac ttcgagaggt ggccggatga agccaagaag tatgatacct cgctctgtct
      301 tgtcatatgc tacggtgccg agttcagact caagagcttg tccgtcgttg ccggcaatgc
      361 cgatgaacga cacaagtgga tcgtcggcct caactggcta gtggaagacc ataaaatctc
      421 aagttaccca agcagactag aatggtggtt acgacgggag ttctacgcca tggggaaaac
      481 aaagaatgat acggtgtcac ttagggacat gaagtcattc atgccatacg tcaacctgaa
```

Figure 5: the ph domain sequence from origin sequence ranging from nucleic acids(26 – 433) with primers are highlighted in yellow
https://www.ncbi.nlm.nih.gov/nuccore/40365362?log$=activity

ISSN: 2776-1010 Volume 3, Issue 2, Feb, 2022

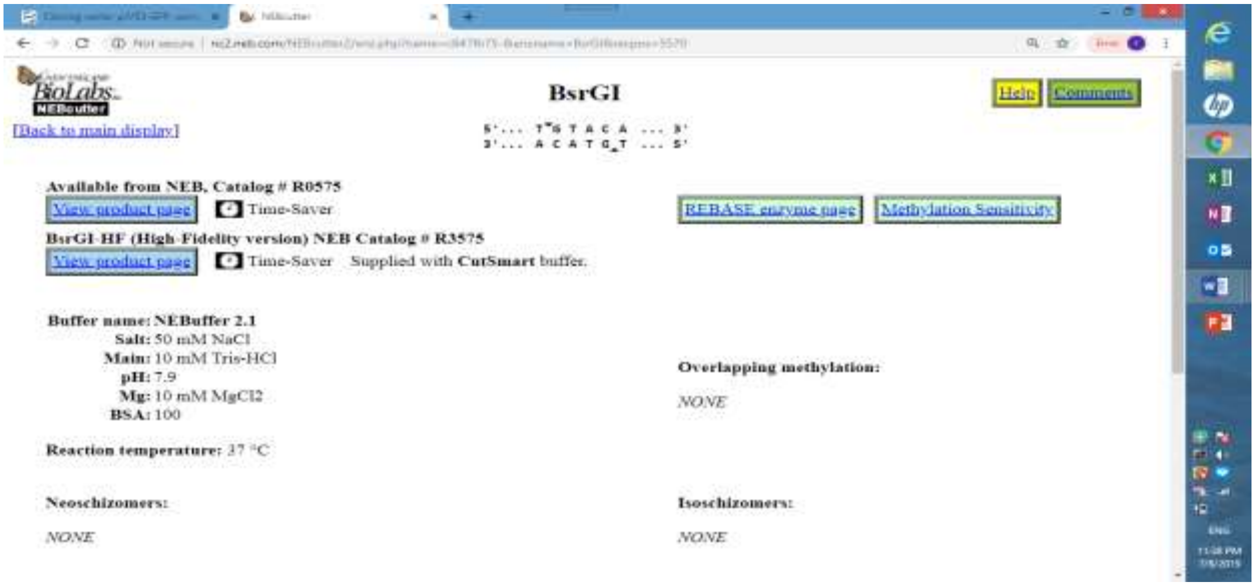


Figure 6: Picture of initial Nebcutter shows the detail of BsrGI restriction enzyme. http://nc2.neb.com/NEBcutter2/enz.php?name=c847fb75-&enzname=BsrGI&recpos=5570

Next , we will used the restriction enzyme BsrGI that cut out the primers match the sites chosen for the ph domain. so GFP – PH fusion protein can be made.

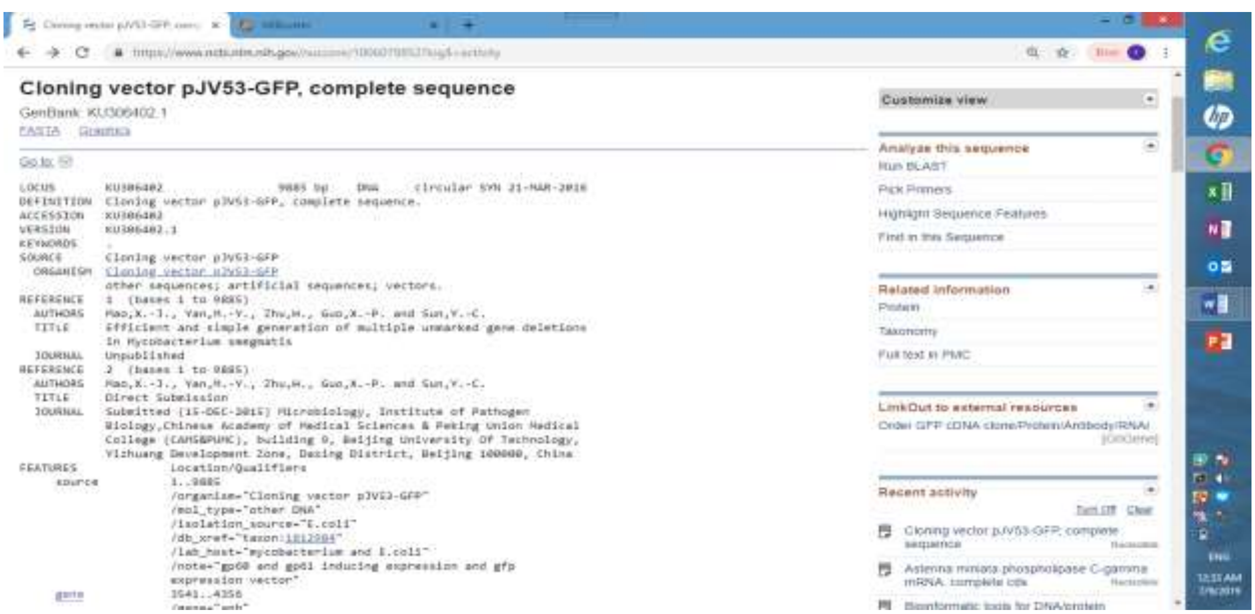


Figure 7 : pjv53-GFP cloning vector DNA sequence found in NCBI.

ISSN: 2776-1010 Volume 3, Issue 2, Feb, 2022

Figure 8: Combined PH domain with pjv53-GFP cloning vector and restriction enzyme highlight green . This sequence was copied and pasted into ORF Finder.
https://www.ncbi.nlm.nih.gov/orffinder/

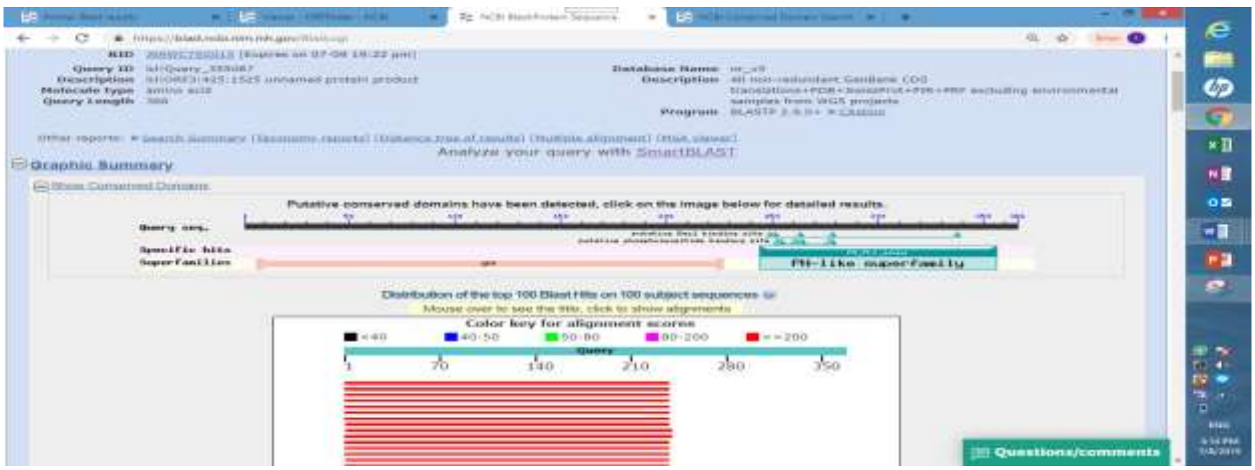


Figure 9:BLAST results showing successful production of GFP-PH fusion protein.
https://blast.ncbi.nlm.nih.gov/Blast.cgi

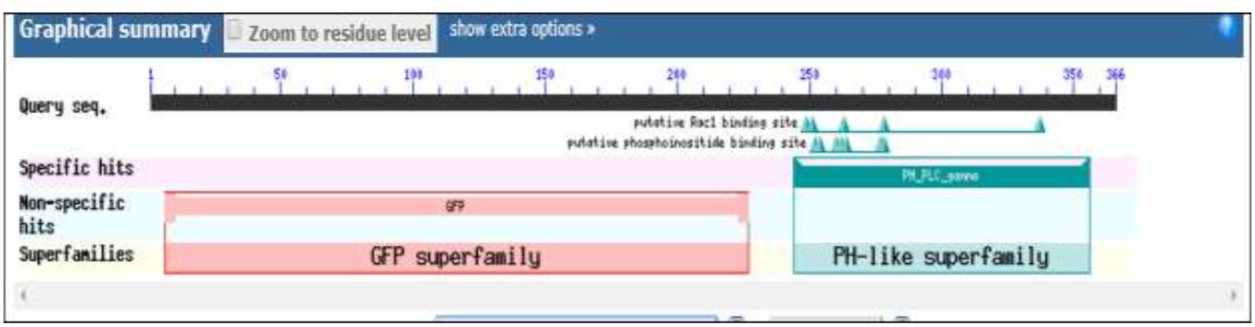


Figure 10: Zoomed-in version of successfully made GFP-PH fusion protein
https://blast.ncbi.nlm.nih.gov/Blast.cgi

ISSN: 2776-1010 Volume 3, Issue 2, Feb, 2022

The egg's Ca2+ levels grow in response to the sperm during fertilization, which is crucial in getting the egg to start developing at the very least, C2+ when IP3 levels rise, it is released from the endoplasmic reticulum is responsible for the rise in C2+ in echinoderm and vertebrate eggs [20] [21]. However, it has not been determined how IP3 is generated during fertilization [22] [23].

A phospholipase C enzyme is responsible for this (PLC), IP3 is generated from PIP2. The enzymes in this group contains δ, γ, and β isoforms. PLC β is activated by G proteins, whereas tyrosine kinases activate PLC [24]. Despite the fact that all three an increase in Ca2+ can trigger PLC isoforms the control of PLC δ remains a mystery, even if the enzymatic activity of all three PLC isoforms may be stimulated by an increase in Ca2+ [25] [26] [27]. One of these phospholipase C isoforms is activated most likely leads in the production of IP3 during fertilization.

Eggs contain PLC γ and PLC β pathway proteins. For example, expression of PLC pathway/ G protein -Dependent receptors such as the serotonin 2c or muscarinic m1 receptors allows for C2+ release in eggs when the appropriate antagonists are used [28] [29]. This implies the presence of functional PLCβ and related G proteins. Exogenous tyrosine kinase/PLC γ receptors, such as those for PDGF or EGF, can be expressed in frog and starfish eggs to allow C2+ release as a result of exposure to these agonists. Ca2+ release is not caused by receptors with a single point mutation that don't activate PLCγ. A functional PLC is evident from these data. These studies have not been done on mammalian eggs, but immunoblotting has shown the presence of PLC γ.

Conclusion

Several prior research have looked into whether PLCγ or PLCβ pathways are responsible for C2+ release during fertilization. Because of concerns about the selectivity of the pharmaceutical inhibitors used, the results of these trials have not been conclusive. To determine whether PLCγ - or PLCβ-mediated Ca2+ release mechanisms are involved in fertilization, we used a recombinant PLC protein component to inject starfish eggs, and it inhibited PLCγ activation but not PLCβ activation.

Acknowledgements

It is matter of honor and privilege for me to offer grateful acknowledgement to my supervisor Dr.Alyaa Abdelhameed for all his invaluable support, superb guidance and encouragement throughout the duration of this study.

References

1. Bianchi, E., & Wright, G. J. (2020). Find and fuse: Unsolved mysteries in sperm–egg recognition. PLoS biology, 18(11), e3000953.
2. Satheeshkumar, R., Zhu, R., Feng, B., Huang, C., Gao, Y., Gao, L. X., ... & Wang, W. L. (2020). Synthesis and biological evaluation of heterocyclic bis-aryl amides as novel Src homology 2 domain containing protein tyrosine phosphatase-2 (SHP2) inhibitors. Bioorganic & Medicinal Chemistry Letters, 30(11), 127170.

ISSN: 2776-1010 Volume 3, Issue 2, Feb, 2022

3. Liu, W., Cai, M. J., Zheng, C. C., Wang, J. X., & Zhao, X. F. (2014). Phospholipase Cγ1 connects the cell membrane pathway to the nuclear receptor pathway in insect steroid hormone signaling. Journal of Biological Chemistry, 289(19), 13026-13041.
4. Aki, S., Yoshioka, K., Takuwa, N., & Takuwa, Y. (2020). TGFβ receptor endocytosis and Smad signaling require synaptojanin1, PI3K–C2α-, and INPP4B-mediated phosphoinositide conversions. Molecular biology of the cell, 31(5), 360-372.
5. Podlewska, S., Bugno, R., Lacivita, E., Leopoldo, M., Bojarski, A. J., & Handzlik, J. (2021). Low basicity as a characteristic for atypical ligands of serotonin receptor 5-HT2. International Journal of Molecular Sciences, 22(3), 1035.
6. Santulli, G., Lewis, D., des Georges, A., Marks, A. R., & Frank, J. (2018). Ryanodine receptor structure and function in health and disease. Membrane protein complexes: structure and function, 329-352.
7. Santella, L., Limatola, N., & Chun, J. T. (2020). Cellular and molecular aspects of oocyte maturation and fertilization: a perspective from the actin cytoskeleton. Zoological Letters, 6(1), 1-21.
8. AL-Amery, M., Fowler, A., Unrine, J. M., Armstrong, P., Maghirang, E., Su, K., ... & Hildebrand, D. (2020). Generation and Characterization of a Soybean Line with a Vernonia galamensis Diacylglycerol Acyltransferase-1 Gene and a myo-Inositol 1-Phosphate Synthase Knockout Mutation. Lipids, 55(5), 469-477.
9. Jacobs, R. S., & Wilson, L. (2020). Fertilized sea urchin eggs as a model for detecting cell division inhibitors. In Modern analysis of antibiotics (pp. 481-493). CRC Press.
10. Kramer, F., Dernedde, J., Mezheyeuski, A., Tauber, R., Micke, P., & Kappert, K. (2020). Platelet-derived growth factor receptor β activation and regulation in murine myelofibrosis. haematologica, 105(8), 2083.
11. Wessel, G. M., Wada, Y., Yajima, M., & Kiyomoto, M. (2021). Bindin is essential for fertilization in the sea urchin. Proceedings of the National Academy of Sciences, 118(34).
12. Campos, S., Troncoso, J., & Paredes, E. (2021). Major challenges in cryopreservation of sea urchin eggs. Cryobiology, 98, 1-4.
13. Stein, P., Savy, V., Williams, A. M., & Williams, C. J. (2020). Modulators of calcium signalling at fertilization. Open biology, 10(7), 200118.
14. Ehm, P. A., Lange, F., Hentschel, C., Jepsen, A., Glück, M., Nelson, N., ... & Jücker, M. (2019). Analysis of the FLVR motif of SHIP1 and its importance for the protein stability of SH2 containing signaling proteins. Cellular Signalling, 63, 109380.
15. Guo, Q., Su, J., Xie, W., Tu, X., Yuan, F., Mao, L., & Gao, Y. (2020). Curcumin-loaded pea protein isolate-high methoxyl pectin complexes induced by calcium ions: Characterization, stability and in vitro digestibility. Food Hydrocolloids, 98, 105284.
16. Jadwin, J. A., Curran, T. G., Lafontaine, A. T., White, F. M., & Mayer, B. J. (2018). Src homology 2 domains enhance tyrosine phosphorylation in vivo by protecting binding sites in their target proteins from dephosphorylation. Journal of Biological Chemistry, 293(2), 623-637.

ISSN: 2776-1010 Volume 3, Issue 2, Feb, 2022

17. Hansen, C. E., Qiu, Y., McCarty, O. J., & Lam, W. A. (2018). Platelet mechanotransduction. Annual review of biomedical engineering, 20, 253-275.
18. Iwatate, R. J., Yoshinari, A., Yagi, N., Grzybowski, M., Ogasawara, H., Kamiya, M., & Nakamura, M. (2020). Covalent self-labeling of tagged proteins with chemical fluorescent dyes in BY-2 cells and Arabidopsis seedlings. Plant Cell, 32(10), 3081-3094.
19. Li, L., Ji, S., Shrestha, C., Jiang, Y., Liao, L., Xu, F., ... & Xie, Z. (2020). p120-catenin suppresses proliferation and tumor growth of oral squamous cell carcinoma via inhibiting nuclear phospholipase C-γ1 signaling. Journal of Cellular Physiology, 235(12), 9399-9413.
20. Paroha, R., Chourasia, R., Rai, R., Kumar, A., Vyas, A. K., Chaurasiya, S. K., & Singh, A. K. (2020). Host phospholipase C-γ1 impairs phagocytosis and killing of mycobacteria by J774A. 1 murine macrophages. Microbiology and Immunology, 64(10), 694-702.
21. Li, C., Liu, F., Liu, S., Pan, H., Du, H., Huang, J., ... & Wei, Y. (2020). Elevated myocardial SORBS2 and the underlying implications in left ventricular noncompaction cardiomyopathy. EBioMedicine, 53, 102695.
22. Hobbs, H. T., Shah, N. H., Badroos, J. M., Gee, C. L., Marqusee, S., & Kuriyan, J. (2021). Differences in the dynamics of the tandem-SH2 modules of the Syk and ZAP-70 tyrosine kinases. Protein Science, 30(12), 2373-2384.
23. Chiarelli, R., Martino, C., & Roccheri, M. C. (2019). Cadmium stress effects indicating marine pollution in different species of sea urchin employed as environmental bioindicators. Cell Stress and Chaperones, 24(4), 675-687.
24. Terrer, C., Jackson, R. B., Prentice, I. C., Keenan, T. F., Kaiser, C., Vicca, S., ... & Franklin, O. (2019). Nitrogen and phosphorus constrain the CO2 fertilization of global plant biomass. Nature Climate Change, 9(9), 684-689.
25. Colombo, N., Sessa, C., du Bois, A., Ledermann, J., McCluggage, W. G., McNeish, I., ... & Zeimet, A. G. (2019). ESMO–ESGO consensus conference recommendations on ovarian cancer: pathology and molecular biology, early and advanced stages, borderline tumours and recurrent disease. Annals of Oncology, 30(5), 672-705.
26. Hu, Q., & Wolfner, M. F. (2019). The Drosophila Trpm channel mediates calcium influx during egg activation. Proceedings of the National Academy of Sciences, 116(38), 18994-19000.
27. Bao, W., Yan, T., Deng, X., & Wuriyanghan, H. (2020). Synthesis of full-length cDNA infectious clones of Soybean mosaic virus and functional identification of a key amino acid in the silencing suppressor Hc-pro. Viruses, 12(8), 886.
28. Zamora, L. N., Delorme, N. J., Byrne, M., & Sewell, M. A. (2020). Lipid and protein utilization during lecithotrophic development in the asteroid Stegnaster inflatus, with a review of larval provisioning in lecithotrophic echinoderms. Marine Ecology Progress Series, 641, 123-134.
29. Li, L., Ji, S., Shrestha, C., Jiang, Y., Liao, L., Xu, F., ... & Xie, Z. (2020). p120-catenin suppresses proliferation and tumor growth of oral squamous cell carcinoma via inhibiting nuclear phospholipase C-γ1 signaling. Journal of Cellular Physiology, 235(12), 9399-9413.