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Bioinformatics Tools Help to find out the Role of Unknown SRFK Initiation on Egg Activation at the Time of Fertilization

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Abstract

Release of Ca^{2+} ions from endoplasmic reticulum indicates egg activation at fertilization time [1]. SRC family kinase protein is directly or indirectly involved Phospholipase C Gamma (Plcγ) in this activation that leads to Ca^{2+} release [1]. Ca^{2+}elevation in egg helps to block polyspermy and helps embryonic development [2]. It is already known that SFK is present in starfish *Asterina miniata* eggs and SFK1, SFK2 are main SRC kinases which are responsible for egg activation [3]. There are several SFK proteins present and perhaps they might have roles in ca^{2+} signalling pathway and egg activation. In this work, our main goal was to find out gene sequences of other unknown SRC kinases those are mainly responsible for egg activation at the time of egg fertilization. To achieve our goal, we use different type of bioinformatics tools from where we come to know gene alignment position, exon part of gene, phylogenic relationship of query protein and function of proteins according to their domain.

Introduction

Ca^{2+} release in all species at fertilization time is a hallmark of egg activation. Ca^{2+} helps to block polyspermy that initiate egg activation. Evidence that Src-type tyrosine kinase activity is necessary for initiation of calcium release at fertilization. SRC kinase is an important component which produces IP. From recent studies, it is found in some lower animals like starfish, sea urchin and frogs that SFK directly activate Plcγ and that produces IP_3. CA^{2+} is released from IP3 receptor of ER. By using different inhibitor of SRC kinase it is proved that ca^{2+} release is delayed when SRC is inhibited and increase of rapidly activation of eggs is happened when kinase activity is increased. SFKs is expressed in starfish (*Asterina miniata*) egg and mainly SFK1, SFK2 and SFK3 are involved egg activation and ca^{2+} release. There are several SFK proteins are present and perhaps they might have role in Ca^{2+} signalling pathway and egg activation [4]. It is possible that different SFKs might have same overlapping role in Ca^{2+} pathway. Here, our main target is to identify gene sequence of SFK s and other unknown protein which might be involved in egg activation by using different bioinformatics tools. From previous study it is known that marine protostomes worm treated with either PP2 to inhibit SFKs or with U73122 to block PLCγ activity. By using different mechanism, it is known that inhibitor significantly reduce post insinuation of polar body. Confocal imaging techniques proof that Ca^{2+} wave block by U73122 but not by PP2, though immunoblot gives false result about PP2 inhibitor [5].

Methodology

The following software's have been used in Bioinformatics analysis in this study

Blast

Blast (Basic Local Alignment Search Tool) is a sequence similarity search tool that can be used to a quickly search sequence database for matches to a query sequence. Several types of blast exits to compare all combination of nucleotide or protein queries against a nucleotide or protein Blast on query sequence. Blast provides e value, statistical information about alignment. Here, we run blast against Patricia miniata model organism. Mainly whole genome shotgun coatings database is used here for optimizing program for blastn of somewhat similar sequence [6].

Spidey

Spidey is an active alignment and splicing NCBI toolkit. Two main objective of this software are

Alignment regardless intron size

Avoid pseudogenes and paralogs

It first aligns sequences and then collect exons only. It can take any genomic or FASTA mRNA sequence. It helps to determine the identity per exon, no of gaps per exon, Overall percentage identity, donor and acceptor site of exon. Here we use this software to delete intron part from aligning mRNA sequence and shot out the exon part that is coding sequence.

Smart Blast

Smart blast is more specific than blast. Smart blast summery provides a unique combining phytogenic tree and graphical overview between query and five matching sequences. Matching is represented by colored coating. Green indicates match from reference species, blue represents matches from no redundant database and yellow indicates query. Smart blast also gives information of conserved domain matches for query. If there are both superfamily matches and specific hit, then it will show specific hit only. It does not represent matches from multi domain model. Here it is mainly used to find phylogenic relationship with query sequence [7-8].

Interpro

Interpro helps to do functional analysis of protein according to their domain, families. It is mainly used in large-scale analysis of proteomes, genomes and metagenomes and it is very helpful to characterise individual protein sequences. Here it is tried to analysis of unknown function of SRC kinase gene sequences after changing it into Fasta protein format by using X blast toolkit. Additional information such as a description, consistent names and Gene Ontology (GO) terms are associated with each entry, where possible that would be helpful in future gene sequence analysis.

Results

Result1

Blast for checking alignment of our query sequence with other reference sequence

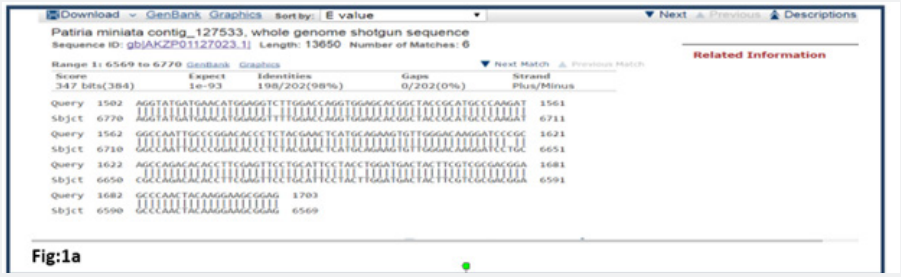

Figure 1A:

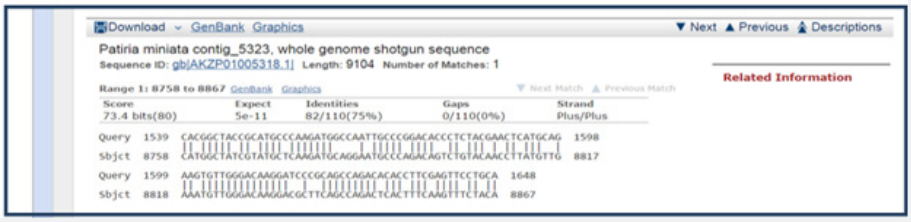

Figure 1B:

Figure1a & Figure 1b: Query sequence is Tyrosine kinase domain(882..1703)of gb|AY518774.1| that aligned with subject sequence gb|AKZP01127023.1|and gb |AKZP01005318.1|.Here 2nd figure (Figure 1b)is less significant than 1st fig (Figure 1a) because in 2nd very short stretch is present where the sequences are aligned. That's why 1st fig result is showed in the next step. Left and right numerical no indicates amino acid position. Above numbers are for query and below numbers are for subject sequence.

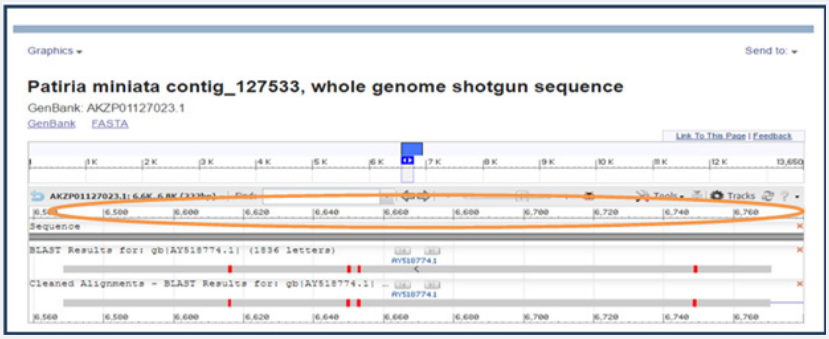

Figure 2: It is a graphical representation of figure 1a. Red portion indicate non-aligned sequences, where query sequences does not match with subject sequence. Circled yellow portion indicate range scale and position. Tools and tracks helps for graphical display.

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BLAST Result for *Asterina miniata* Src family tyrosine kinase (SFK1) mRNA, complete cds (Gene Bank: AY518774.1) (Figure 1a, 1b, & Figure 2).

Misc. feature: 882...1703

/gene="SFK1"

/note="Region: tyrosine kinase domain"

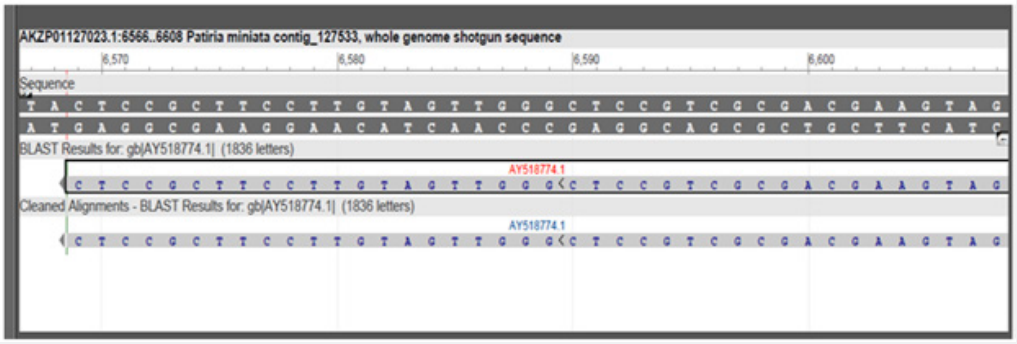

Figure 3a:

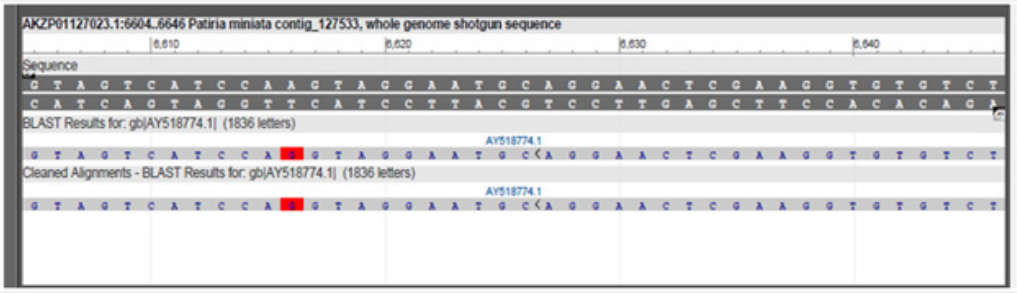

Figure 3b:

Figure 3c:

Figure 3d:

Figure 3a-3d: our query sequence(tyrosine kinase domain of SFK1(gb|AY518774.1|)align with subject sequence range of amino acid 6569 to 6770 of gb|AKZP01127023.1|.Red indicates miss match bases.3a to 3d fig represent continuation of our query sequence in 6569 to 6770 range that is shown after doing zoom in.

Result 2

Scanning exon part of aligned sequence after using Spidey tool (Figure 3a, 3b,3c, 3d).

Result 3

Smart blast of accession AY518774.1for find out photogenic relationship of our query protein SRFk1(Figure4a,4b&Figure5).

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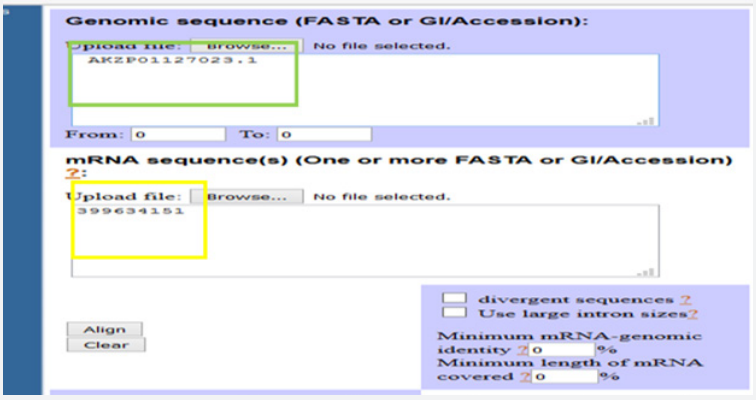

Figure 4a: It represents web page of Spidey software tools which is used for splicing. Green colored box indicate our input accession no and yellow colored box indicate GI no. Here, as our sequence identity is near 98%. So, we can use both subject sequence id or our query sequence id. We will get same result in both cases .After doing blast, by using Spidey tool we can directly separate exon part of long aligned query sequence.

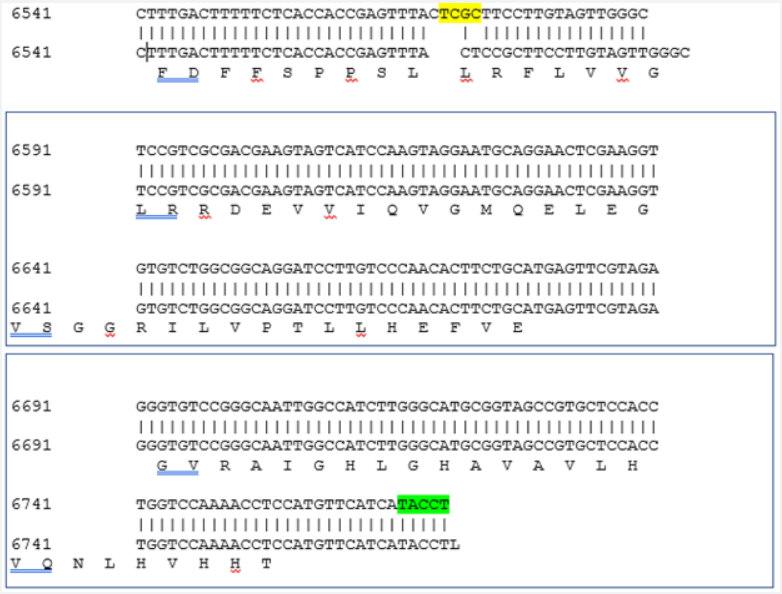

Figure 4b: Blue colored box indicates exon part of aligning mRNA sequence after using speedy software.

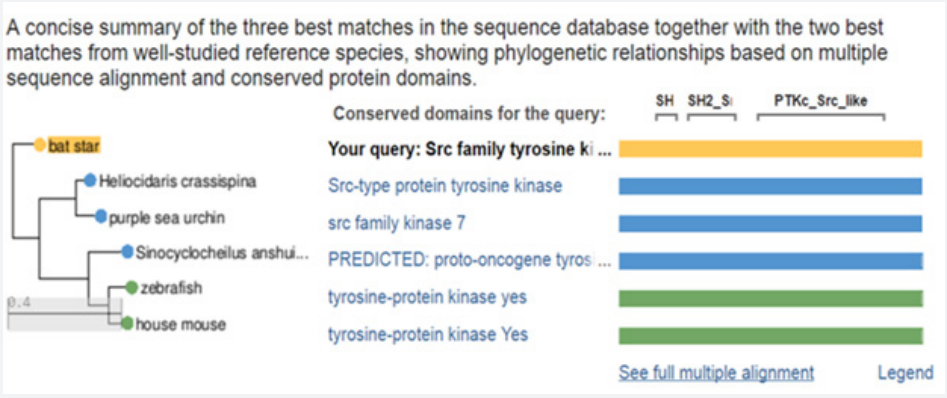

Figure 5: Query species for this search is highlighted in yellow. Green indicates the reference database or subject database. Blue indicates matches from the non-redundant (nr) database. The query for this search is AY518774.1. It represents photogenic relationship with *Asterina miniata* Src family tyrosine kinase (SFK1) with other species.

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Result 4

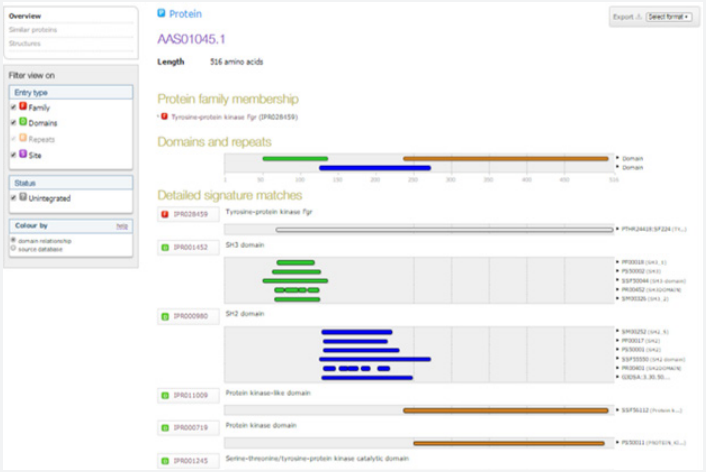

Figure 6a:

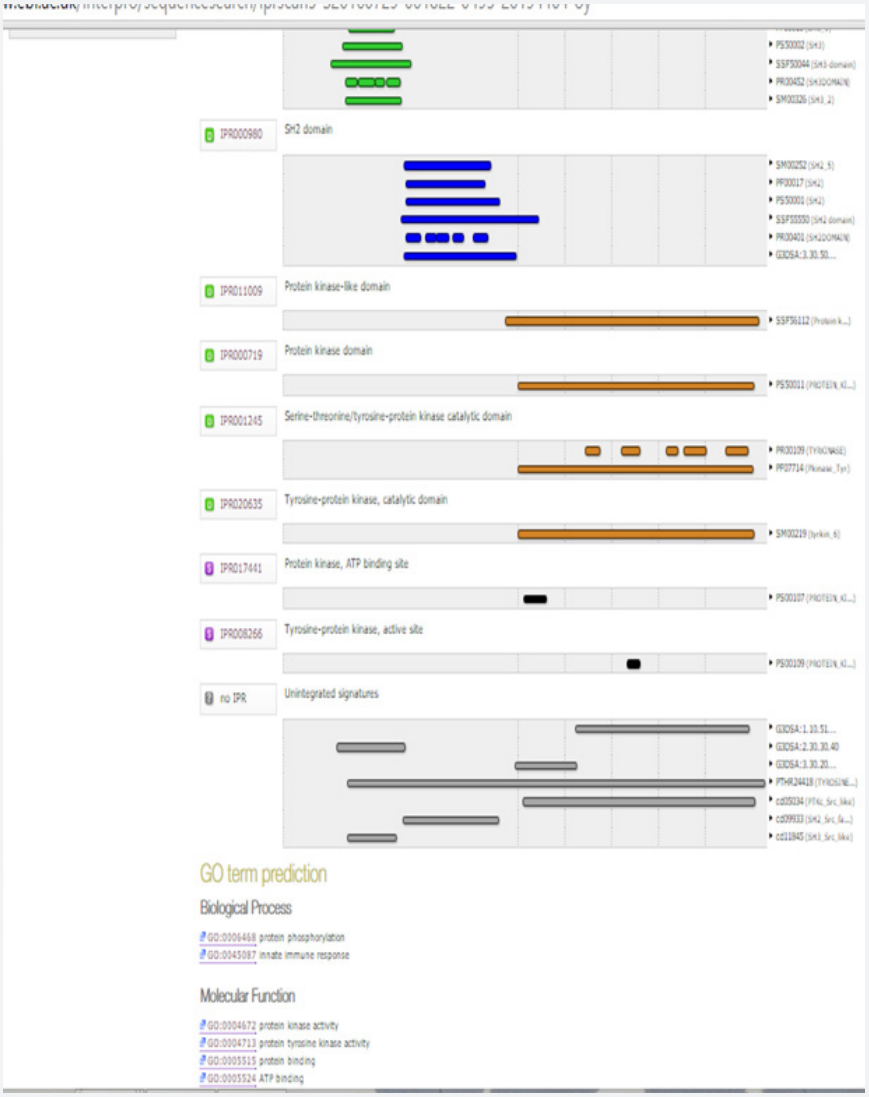

Figure 6b: Functional analysis of protein by using Interpro tools of Src family kinase [Patiria miniata] Sequence ID: gb|AAS01047.1|. It gives the details information about family, domain, repeat site of protein sequence. Green, blue colour, brown, black bar progressively represents SH3, SH2, kinase and ATP binding site. It gives detail information of molecular function.

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Result from Interpro (Figure 6a,6b).

Discussion

In this work *Asterina miniata* Src Family Tyrosine Kinase (SFK1) mRNA, complete cds and *Asterina miniata* Src Family Kinase (SFK3) mRNA, complete cds are mainly used. We already know SFK1 and SFK3 both are important Ca^{2+} signalling pathway and egg activation at fertilization time. By using different bio informatics tool and method we try to find out coding gene sequence, unknown function, alignment range, phytogenic tree and graphical overview of known sequence. This data will be helpful to scan specific gene sequences from other unknown SRC kinase protein that might have role for egg activation and Ca^{2+} signalling pathway at fertilization time.

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