

Oreochromis niloticus- a biomonitoring tool for assessment of trace metals of Theroor wetland, Kanniyakumari district, India

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Abstract - Currently wetland resources for being a significant source of fish production are receiving great attention all over India. One such environmental and economic important wetland in Kanniyakumari district, the Theroor wetland which is now subjected to many of environmental changes due to influx of huge amount of discharges originated from different sources and as well as due to many human activities. This kind of action has transferred various toxic and risky elements into the food chain causing hygienically serious issues like contaminating the water of wetland and at higher concentration acting negatively on macro vertebrate- fish. Most of the metal ions which has the capability to bioaccumulate in aquatic organism at higher concentration may cause biochemical alteration finally leading to mortality. Due to the high sensitivity nature of the aquatic organisms towards the dissolved toxicants the fresh water fish *Oreochromis niloticus* collected from the three sectors (Eastern, Central, and Western) of the wetland has been utilized in the present study as biological indicator/ biomarker to evaluate the impact of metal toxicants at molecular level using proteomic tools. Thus, with the above context the present study was designed to evaluate some metal pollution in Theroor wetland using selected bioinformatic tools to give an overview of impact of metal through proteomic studies. The values of heavy metals (Ni, Hg and Zn) measured in the fresh water fish muscles of *Oreochromis* proven to be baseline data indicating the need for pollution monitoring in wetlands.

keywords - Wetland, Heavy metals, Fresh water fish, Proteomics

I. Introduction

Fresh water plays a vital role in the proper functioning of earth's ecosystem. Any change in fresh water quality will adversely affect the living organisms or make it undeniable. So, many limnologists and toxicologist showed great interest toward measuring water quality of different water bodies around the world. Schuurmann and Markert, (1998) through their research stated that all almost all the pollutants are discharged into the environment every day routinely by humans. Of which, heavy metals are regarded as one of the most serious pollutants of the aquatic environment because of their environmental persistence and tendency to accumulate in aquatic organisms. The relation between aquaculture and pollution is a complex one. Animals living in a water body are affected by changes in the chemical and physical quality of that water. As, the living medium of fish is getting deteriorated, with the general idea to protect fresh water bodies, their quality and fisheries, the expansion of research has been carried to analyze water quality and their relationship with biological productivity and fish because these features serves as a basis for the richness of aquatic environment.

Failler,2006; De Silva and Davy,2010 stated that basically, food from aquatic resources is an important dietary component in many countries and its demand will continue in the forthcoming years. Recent research articles also state that globally consumption of fish by human is about 80-90% as it has high protein content which too supported the reports of Failler, (2006); De Silva and Davy, (2010). Currently due to rapid industrial development, urbanization, construction and deforestation most of the countries are facing environmental problem especially water pollution across the globe. The other common hazardous material which caused negative impacts to the aquatic ecosystem is the heavy metals. Concentration of some heavy metals like Ni, Hg and Zn, etc. induce changes also in morphology, physiological and biochemical parameters in fish.

Fish consumption has increased in importance among health-conscious because it provides a healthy, low cholesterol source of protein and other nutrients including omega-3 (n-3) fatty acid that reduce cholesterol levels and the incidence of heart disease, stroke, and preterm delivery. Besides playing a central physiological role and as heat homeostasis muscles present itself as a crucial metabolic tissue that integrates various biochemical pathways Abdul-Ghani and De Fronzo, (2010); Surajit *et al.* (2011). So fish muscle was chosen as a sample source for proteomic studies in the present research analysis because it is the most edible part of fish and the largest organ system Johnston, (1999); Addis *et al.* (2010) constituting 40-50% of the total body weight, rich in vitamins, minerals, all essential amino acids in right proportions it can help to identify proteins and enzymes that are responsible for increasing meat yield, the commercially important fish vitamins and unsaturated fatty acids, as well as, treatment of fish diseases.

As, proteomics has emerged as a powerful tool for the study of biological systems this technology has been increasingly used during the last years to address different questions related to fish biology. Besides adding to the existing knowledge base on comparative muscle proteomics, the information generated would also serve as the baseline proteogenomic information on this exotic species *Oreochromis niloticus*, the freshwater fish of the family: *Cichlidae* inhabiting in the tropical wetland water bodies of Indian subcontinent. *Oreochromis niloticus* is the main species of freshwater fishes that inhabit in almost all wetlands

of Indian continent is one of the most popular, cheapest and is an important food fish with high nutritive value. This study was conducted to investigate the tissue accumulation of Ni, Hg and Zn, in the edible part, of *O. niloticus* from Theroor wetland and to determine the relationship between the current aquatic contamination with heavy metals and the health hazards to fish consumers from higher tropic levels.

II. MATERIALS AND METHODS

The proteins and enzymes in *Oreochromis niloticus* that are responsible for heavy metal assimilation were identified at the genome level. The proteins with already known function were explored by text mining whereas the functions of hypothetical and uncharacterized proteins were predicted. The KEGG Orthology (KO) system is a pathway based definition of orthologous genes Moriya *et al.* (2007). The function of missing proteins was predicted according to the methods described by Chellapandi, (2011). Briefly, a protein whose function was not yet reported in databases or annotated function not compiled in KEGG pathway database, but which has separate KO system in the KEGG GENES database, their protein function was assigned to be predicted with KO

2.1 Proteome retrieval

The complete proteome of *Oreochromis niloticus* was retrieved from Uniprot Knowledgebase. UniProt KB/Swiss-Prot is the manually annotated and reviewed section of the UniProt Knowledgebase (UniProt KB) (The UniProt Consortium).

2.2 Metabolic Pathway Analysis:

The metabolic pathway analysis of *Oreochromis niloticus* was carried out using KEGG (Kyoto Encyclopedia of Genes and Genome) (Kanehisa *et al.* 2016). KEGG is a database resource that integrates genomic, chemical and systemic functional information. Further, Protein-Protein interactions of *Oreochromis niloticus* can be identified using STING database (Szklarczyk *et al.* 2015).

2.3 Cell simulation

Simulation of intervention of heavy metals in the system of *Oreochromis niloticus* was carried out using Cell designer software (Funahashi *et al.* 2008). It is an application in which compartmental topology, geometry, molecular characteristics, and relevant interaction parameters can be used defined. It automatically forms a corresponding mathematical system of ordinary and/or partial differential equations from the given biological description.

III. Results and Discussion

Wetlands facing major environmental problems associated with the dispersal or disposal of agricultural, industrial and urban wastes generated by human activities. So, wetland throughout the globe has recently been receiving a great attention because of its environmental and economic importance mainly for being a significant source of fish production. Presently due to industrial and technological development many hazardous byproducts are produced which are directly influxed into the waterbodies without undergoing bio-remedial treatments. Discharge of these toxic heavy metals into wetlands ecosystems will alter the physiology of aquatic species and change diversity (both qualitatively and quantitatively) due to their toxicity, persistence and accumulative behavior.

As a result of natural processes and influx of contaminants from human activities the non-biodegradable toxicants, heavy metals enter into the aquatic medium. Fresh water fishes, the most widely distributed aquatic organism due to their continuous contact with the water medium are popular sentinel species used in monitoring aquatic bodies and in determining the resident's diet. Because toxicity with heavy metals will disrupt the function of essential biological molecules such as protein and enzymes in fish via ingestion, absorption on tissues, surface and food chain.

Presently the demand for aquatic resource has further got worsened by increasing population growth leading to inherent food demand specifically protein rich food i.e. fish. Following major issue for the demand in aquatic resource is the environmental pollution in specific, heavy metal pollution. Vidali, (2001); Shukor *et al.* (2006) through their research work stated that bioremediation, the most low-cost method capable of degrading environmental pollutant still have some limitations and controversial such as the inability to degrade heavy metals. So, to protect the aquatic resources like water and fish preventive measures is crucial.

As an alternate to the traditional methods of remediation currently biologist consider proteomics as reliable tool to evaluate the biological response towards the environmental risk so that preventive measures can be taken as it is the best method to elucidate the level of pollutant in aquatic organisms at molecular level. According to Liu *et al.* (2013) proteomics is a well-established technique in the post- genomic era, which deals with large scale expression of proteins in an organism. This being a powerful tool not only describes complete protein changes in any organisms but also helps in comparing variations in protein profile of an organisms at organ, tissue, cell and organelle levels under different stress condition including heavy metals. It is also well known that proteins are the important bio molecule that directly takes part in any organism's stress response.

Moreover, organisms adapting to heavy metals stress are always accompanied with proteomic changes. Thus, proteomic analyses offer a new platform for identifying target proteins which take part in heavy metals detoxification, and in studying complex biological processes and interactions among the possible pathways that involves a network of proteins. Thus, proteomic technique has been subjugated in this research work for deciphering the possible intervention between protein abundance and stress adaptation as it can contribute to better understanding of physiological mechanisms under heavy metal stress and further signaling cascade that leads to changes in the expression to large number of genes in metabolic profile under heavy metal toxicity.

Since heavy metals accumulate through different organs of aquatic organism because of its affinity they are analysed for the contaminants. As muscle is main part of the fish that is consumed by human being, therefore in the present study it is considered the vital part to analyze. With this context in the present work muscle protein of *Oreochromis niloticus* has been analyzed for intervention of metals which will be beneficial for humans in drug designing and maintain food care from consumer point of view. On analysis a total of 3 proteins were identified in *Oreochromis niloticus* involved in nickel assimilation. The list of proteins is tabulated in Table (1) includes 1, 2-dihydroxy-3-keto-5-methylthiopentene dioxygenase, Voltage-dependent T-

type calcium channel subunit alpha, and another Voltage-dependent T-type calcium channel subunit alpha. The KO assignment for these proteins reveals its role in calcium Signaling pathway which is portrayed in Fig 1. From Table 2 it can be inferred that the concentration of the protein I3K0Q7 exhibit positive correlation with the concentration of nickel. But, the concentration of the proteins, I3KJP6 and I3KUE6 decreases with increasing concentration of nickel at various time intervals.

Only one protein was identified in *Orochromis niloticus* involved in mercury assimilation. The details of the protein is tabulated in Table 2 convey that although it is an uncharacterized protein, its function was predicted to be involved in detoxification of mercury. The KO assignment for the protein reveals its role in Choline metabolism in cancer which is portrayed in Fig 2. From Table 3 it can be inferred that the concentration of the protein I3KKS1 exhibit positive correlation with the concentration of mercury at various time intervals. A total of 4 proteins were identified in *Orochromis niloticus* involved in zinc assimilation. The list of proteins is tabulated in Table 4 includes lactin receptor, ATPase asna1, E3 ubiquitin-protein ligase parkin and uncharacterized protein. The KO assignment for these proteins reveals its role in protein processing pathway which is portrayed in Fig 3. From Table 5 it can be inferred that the concentration of the proteins Q91513 and I3KRS7 exhibit positive correlation with the concentration of zinc. But, the concentration of the proteins, I3JF6 and I3JAW4 decreases with increasing concentration of zinc at various time intervals.

The heavy metals considered most toxic and apparently most poisonous to freshwater life which are reviewed in the present study include zinc, nickel and mercury. These heavy metals get accumulated in the muscles, bones, blood and fat. On consumption of these toxic aquatic organisms young children and adults are especially delicate to even low levels of heavy metals. In contrast, the present study reveals the role of nickel in calcium signaling pathway, it was already confirmed that nickel exposure results in decreased blood parameters in fishes Gochfeld, (2003). But mercury contamination causes irreversible damages, such as neurological impairment and lesions, behavioral and cognitive changes, ataxia, as well as convulsions, in addition to its harmful effect on reproduction which supports the present finding of the role of mercury assimilation system in cancer Oliveira, (2006). In contrast, zinc exposure has been shown to induce histopathological alterations in ovarian tissue of *Tilapia nilotica* (degeneration and hyperaemia) Abd, (1999) and liver tissue of *Oreochromis mossambicus*, but the present study shows its involvement in protein processing as it's a cofactor for many enzymes. Thus, the present proteomic technique used in the present study have received a great deal of attention because they not only offer the potential to unravel novel mechanisms of toxic action but are also support to the discover biomarkers for exposure and their effects.

IV Conclusion

The consumption of the economically important *O. niloticus* from study area considered to pose a health hazard on human health. The present results also showed that, the rate of change in the levels of metal bioaccumulation imposing the protein alteration in fish on consumption by local inhabitant of Theroor will be unsafe. In addition, cooperation between different authorities and efforts are needed to protect these precious food web aquatic components to sustain the biodiversity in wetlands.

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Table 1 Proteins involved in nickel assimilation in *Oreochromis niloticus*

S. No	Uniprot ID	Protein	Gene name	Reaction	Function
1	I3K0Q7	1,2-dihydroxy-3-keto-5-methylthiopentene dioxygenase	Adi1	1,2-dihydroxy-5-(methylthio)pent-1-en-3-one + O ₂ = 4-(methylthio)-2-oxobutanoate + format.	Nickel binding
2	I3KJP6	Voltage-dependent T-type calcium channel subunit alpha	-	-	Calcium Channel blocked by Nickel
3	I3KUE6	Voltage-dependent T-type calcium channel subunit alpha	CACNA1G	-	Calcium Channel blocked by Nickel

Table 2 Rate of change of nickel assimilation system at various concentrations of nickel in *Oreochromis niloticus*

Conc. of Nickel	Conc. in mM			Time in Min
	I3K0Q7	I3KJP6	I3KUE6	
2.09	90	15	19	5
2.08	85	17	29	10
2.09	83	18	36	15
1.8	81	25	47	20
1.79	85	29	58	25
1.72	74	33	63	30
1.52	69	39	72	35
1.49	58	42	79	40
1.45	47	49	83	45
1.98	36	55	89	50
1.95	28	63	92	55
1.89	19	72	95	60
Correlation	0.157854	-0.27649	-0.48913	

Table 3 Proteins involved in mercury assimilation in *Oreochromis niloticus*

S. No	Uniprot ID	Protein	Gene name	Reaction	Function
1	I3KKS1	Uncharacterized protein	LOC100709187	-	detoxification of mercury ion; transmembrane transporter activity

Table 4 Rate of change of mercury assimilation system at various concentrations of mercury in *Oreochromis niloticus*.

Conc. of Mercury	Conc. in mM	Time in Min
	I3KKS1	
0.005	95	5
0.004	90	10
0.004	85	15
0.002	87	20
0.002	82	25
0.002	74	30
0.001	63	35
0.001	59	40
0.001	46	45
0.003	39	50
0.003	27	55
0.002	19	60
Correlation	0.400045	

Table 5 Proteins involved in Zinc Assimilation in *Oreochromis niloticus*.

S. No	Uniprot ID	Protein	Gene name	Reaction	Function
1	Q91513	Prolactin receptor	Prlr	-	Zinc metal binding
2	I3KRS7	ATPase asna1	Asna1	-	Zinc metal binding
3	I3JF6	E3 ubiquitin-protein ligase parkin	Park2	S-ubiquitinyl-[E2 ubiquitin-conjugating enzyme]-L-cysteine + [acceptor protein]-L-lysine = [E2 ubiquitin-conjugating enzyme]-L-cysteine + N(6)-ubiquitinyl-[acceptor protein]-L-lysine	Zinc metal binding
4	I3JAW4	Uncharacterized protein	Vps11	-	Zinc ion binding

Table 6 Rate of change of zinc assimilation system at various concentrations of zinc in *Oreochromis niloticus*.

Conc. of Zinc	Conc. in mM				Time in Min
	Q91513	I3KRS7	I3JF6	I3JAW4	
1.27	45	95	25	5	5
1.29	43	86	29	16	10
1.14	42	82	35	23	15
0.74	38	79	39	39	20
0.75	35	74	42	45	25
0.66	22	69	45	57	30
0.54	29	65	47	63	35
0.51	32	61	58	77	40
0.42	31	55	62	82	45
1.18	21	52	68	91	50
0.98	22	48	74	95	55
0.97	15	44	79	99	60
Correlation	0.263531	0.358249	-0.25968	-0.41141	

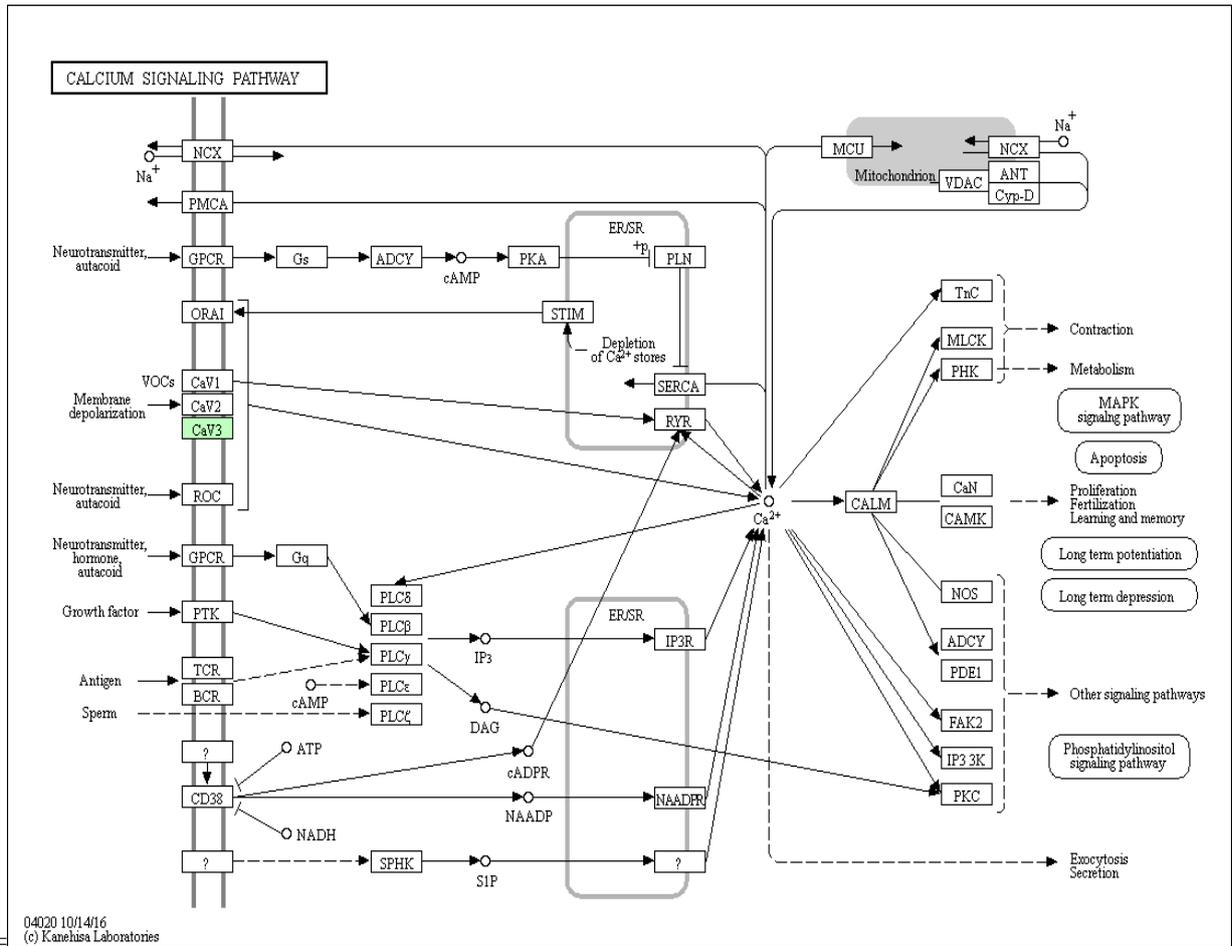


Fig 1 Network of calcium signaling pathway in *Oreochromis niloticus*

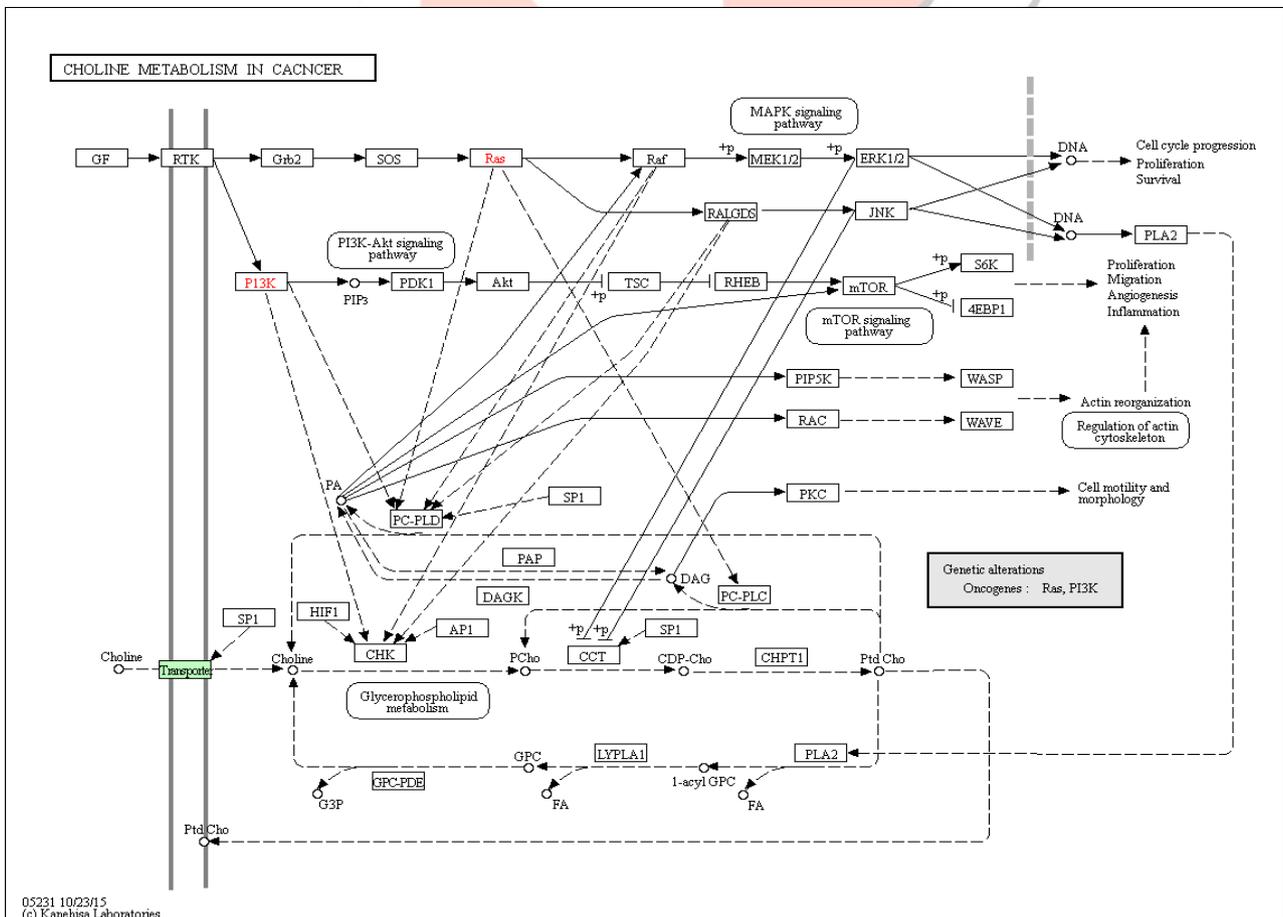


Fig 2 Network of Choline metabolism in *Oreochromis niloticus*

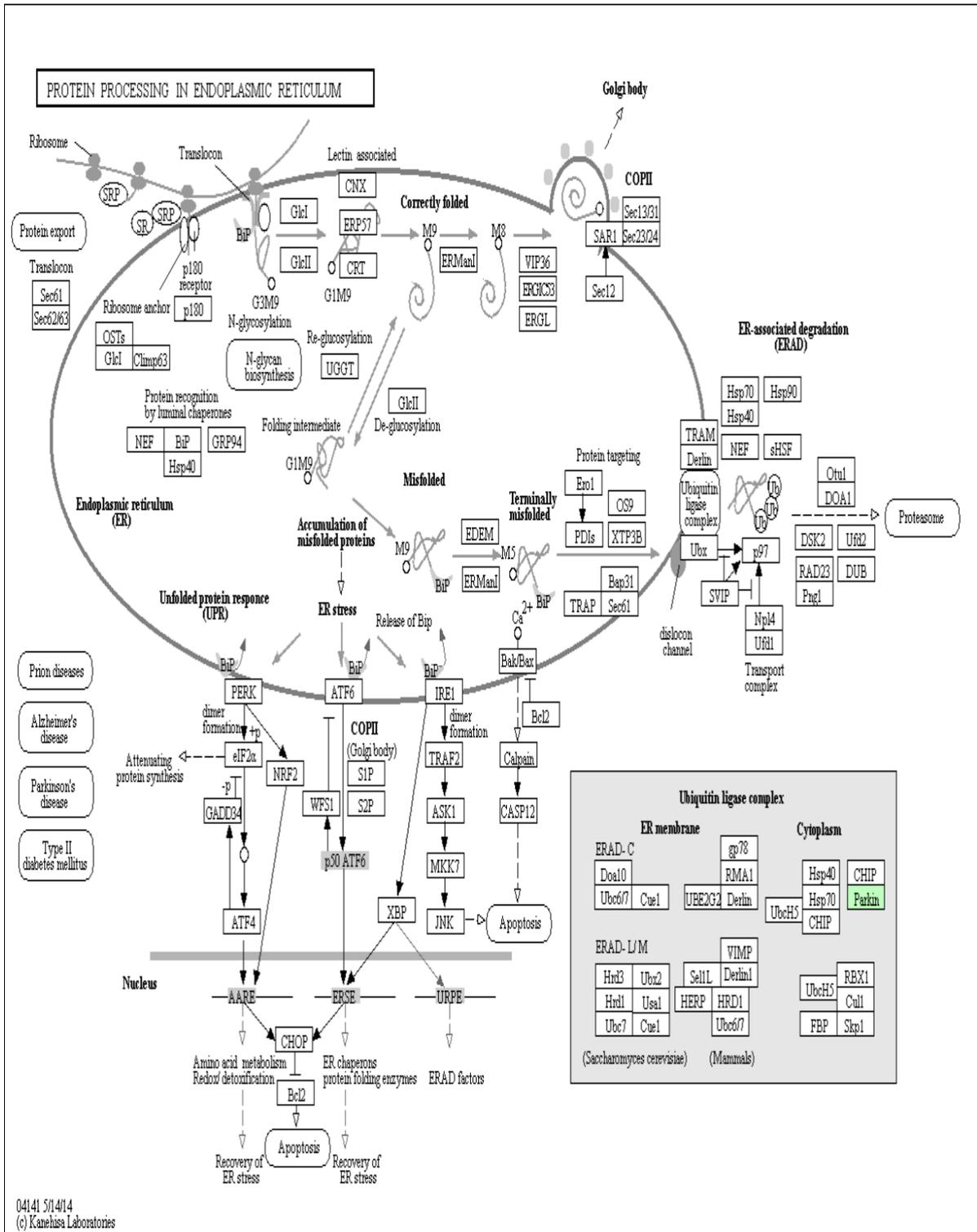


Fig 3 Network of protein processing in endoplasmic reticulum in *Oreochromis niloticus*