

神戸大学バイオシグナル総合研究センター共同利用研究報告書

平成 29年 4 月30 日

神戸大学バイオシグナル総合研究センター長 殿

所属機関・部局名 京都産業大学 総合生命科学部  
 職 名 研究員  
 研究代表者名 Tokmakov Alexander A.

下記のとおり平成28年度の共同利用研究成果を報告します。

記

(課題番号: 281027 )

1. 共同利用研究 課題名	Postovulatory ageing of <i>Xenopus</i> eggs			
2. 共同利用研究 目的	The study is aimed at the investigation of intracellular processes that occur upon ageing of <i>Xenopus</i> frog eggs. The focus will be set on the mechanisms of spontaneous activation and meiotic exit in mature metaphase-arrested eggs awaiting fertilization.			
3. 共同利用研究 期間	平成 28年 07月 01日 ~ 平成 29年 03月 31日			
4. 共同利用研究組織				
氏 名	所属部局等	職名等	役割分担	
(研究代表者) Tokmakov A.A.	京都産業大学 総合生命科学部	研究員	80%	
(分担研究者) Ijiri Takashi W.	摂南大学 理工学部 生命科学科	講師	20%	
5. センター内受入研究者	研究部門・ 分野名	Signal Function ・ Cell proliferation and differentiation	氏 名	Iwasaki Tetsushi

※ 次の6, 7, 8の項目は、枠幅を自由に変更できます。但し、6, 7, 8の項目全体では1頁に収めて下さい。

6. 共同利用研究計画

The following tasks were planned to be solved during the project:

- Set up of a fluorescent method for detection of  $(Ca_i^{2+})$ . Monitoring  $(Ca_i^{2+})$  in ageing eggs.
- Detection of CaMKII, MAPK activation state by immunoblotting.
- Monitoring of ATP levels in ageing *Xenopus* eggs.
- Set up of a fluorescent method for evaluation of the mitochondrial membrane potential. Monitoring mitochondrial membrane potential in ageing *Xenopus* eggs.
- Set up of a fluorescent method for detection of ROS. Monitoring ROS levels in ageing *Xenopus* eggs.
- Effect of calcium chelators, protein kinase inhibitors and ROS scavengers on postovulatory ageing.

7. 共同利用研究の成果

A new fluorescent method for detection of ROS in *Xenopus* oocytes and eggs has been set, based on the use of cell permeant dye dichlorofluorescein diacetate (DCFDA). Monitoring of ROS levels in *Xenopus* eggs and oocytes revealed accumulation of cytoplasmic ROS with age. The capacity of different antioxidants (AOX) to modulate intracellular ROS levels was investigated and the most effective AOXs were pinpointed for further studies.

A fluorescent method of  $Ca_i^{2+}$  detection in *Xenopus* oocytes and eggs was developed, based on the use of Fluo-8 calcium assay kit (Abcam). Levels of intracellular  $Ca_i^{2+}$  were monitored in resting oocytes and eggs and the cells activated with  $H_2O_2$ . Various patterns of calcium signal were observed in these models, reflecting their different responsiveness to the activation stimulus.

Quantitative assessment of SA- $\beta$ -gal, a marker of cell senescence, has been performed in aging oocytes and eggs. Lysosomal localization of the enzyme was demonstrated. Gradual increase of the lysosomal SA- $\beta$ -gal activity was observed in aging oocytes and eggs. For the first time, a new cell-permeable self-ligating fluorescent substrate, SPiDER- $\beta$ -gal, was successfully employed to investigate SA- $\beta$ -gal activity in *Xenopus* oocytes and eggs.

8. 共同利用研究成果の学会発表・研究論文発表状況

(本センターの担当教員の氏名の記載, 又はこの共同利用研究に基づくとの記載のある論文等を記載して下さい。なお, 論文の場合は, 別刷りを1部提出してください。)

Tokmakov AA, Iguchi S, Iwasaki T, Sato K. (2016) Analysis of ROS levels in aging *Xenopus* oocytes and eggs. 87th Annual Meeting of the Zoological Society of Japan, November 17, 2016, Ginowan, Japan.

Tokmakov AA, Surawich J, Sato K. (2016) Quantitative assessment of cell senescence markers in aging *Xenopus* oocytes and eggs. 39<sup>th</sup> Annual Meeting of MBSJ, December 02, 2016, Yokohama, Japan.

Ijiri TW, Kawano N. Organizers of section: "In Focus: Organelles in Sperm and Oocytes." 87th Annual Meeting of the Zoological Society of Japan, November 17, 2016, Ginowan, Japan.

Tokmakov AA, Iguchi S, Iwasaki T, Sato K, Fukami Y. (2017) Global decay of mRNA is a hallmark of apoptosis in aging *Xenopus* eggs. *RNA Biol.*14:339-346.

9. 共同利用研究に関連した受賞, 博士学位論文の取得, 大型研究プロジェクトや競争的資金の獲得等がありましたらご記入ください。