

様式3

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 31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 30 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 is 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 30年 4月 01日 ~ 平成 31年 3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。

(課題番号:281027)

## 6. 共同利用研究計画

The following tasks were pursued during this year of study:

- Detection of SA- $\beta$ -gal activity in ageing *Xenopus* eggs and oocytes;
- Studies of subcellular localization of SA- $\beta$ -gal in the oocytes and eggs;
- Fluorescent detection of  $Ca_i^{2+}$ . Monitoring  $Ca_i^{2+}$  in ageing *Xenopus* eggs and egg extracts;
- Monitoring ATP levels in ageing and overactivated *Xenopus* eggs;
- Evaluation of lysosomal acidification in ageing eggs by a fluorescent method;
- Effect of calcium chelators and protein kinase inhibitors on postovulatory ageing of *Xenopus* oocytes and eggs.

## 7. 共同利用研究の成果

During this year, we conducted biochemical and microscopic analyses concerning localization of senescence-associated  $\beta$ -galactosidase (SA- $\beta$ -gal) in *Xenopus* oocytes and eggs. SA- $\beta$ -gal activity was found to be located mainly within a fraction of dense cytoplasmic granules that were extensively stained with the lysosome-specific dye LysoTracker. The granules were abundant in the cytoplasm and they had an average size of  $8.9 \pm 5.6 \mu\text{m}$ . These properties allowed us to identify the SA- $\beta$ -gal-containing granules as a subpopulation of yolk platelets, specialized late endosomes that accumulate and store processed vitellogenin in frog oocytes. Further analysis revealed an increase of SA- $\beta$ -gal activity in *Xenopus* eggs, but not oocytes, aged *in vitro* over 48 hours. Our results suggest that acidification of the endosomal compartment during egg aging is responsible for this increase. In the course of this study, an optimized method has been developed for detection of SA- $\beta$ -gal in *Xenopus* oocytes and eggs, using the recently developed cell-permeable fluorescent substrate of  $\beta$ -galactosidase, SPiDER- $\beta$ -gal.

We also studied effects of the calcium chelators, such as EGTA, BAPTA, BAPTA-AM, on hydrogen peroxide-induced egg overactivation. Protein and energy homeostases were investigated in the overactivated eggs based on measurements of intracellular ATP and cytosolic protein contents. Time-dependent lipofuscin accumulation was observed in the hydrogen peroxide-treated *Xenopus* 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, Sato KI. (2019) Reconstitution of extracellular calcium signaling in *Xenopus* egg extracts. *Methods Mol Biol* 1920:41-57.

Sato KI, Tokmakov AA. (2019) Membrane Microdomains as Platform to Study Membrane-Associated Events During Oogenesis, Meiotic Maturation, and Fertilization in *Xenopus laevis*. *Methods Mol Biol* 1920:59-73.

Tokmakov A.A., Sato KI. (2019) Activity and intracellular localization of senescence-associated  $\beta$ -galactosidase in aging *Xenopus* oocytes and eggs. *Exp Gerontology* 119:157-167.

Tokmakov AA, Sato KI. (2018) Detection of SA- $\beta$ -gal activity in *Xenopus* oocytes using SPiDER- $\beta$ -gal. The 41<sup>st</sup> Annual Meeting of the Molecular Biological Society of Japan, November 30<sup>th</sup>, 2018, Yokohama, Japan.

Isobe T., Sato K., Yoshida H., Awamura M., Hokatsu I., Matsumoto Y., Sato K-I., Tokmakov A. (2018) *In vitro* reconstruction of oocyte life cycle: from ovulation to apoptosis. November 30<sup>th</sup>, 2018, Yokohama, Japan.

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。