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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 30 年 04 月 16 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29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 29 年 04 月 01 日 ~ 平成 30 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 pursued during this year of study:

- Fluorescent detection of (Ca_i^{2+}). Monitoring (Ca_i^{2+}) in ageing, activated and hyperactivated eggs;
- Detection of calpain and cathepsin activity in ageing, activated, and hyperactivated eggs;
- Monitoring of ATP levels in ageing *Xenopus* eggs;
- Evaluation of the mitochondrial membrane potential by a fluorescent method, monitoring mitochondrial membrane potential in ageing *Xenopus* eggs;
- Detection of intracellular 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 fluorescent method of Ca_i^{2+} detection in *Xenopus* oocytes and eggs based on the use of Fluo-8 indicator was employed to monitor levels of intracellular Ca_i^{2+} in resting oocytes and eggs, and the cells activated or hyperactivated with H_2O_2 . Various patterns of calcium signal were observed in these models. It was found that egg hyperactivation led to a prolonged uncompensated release of intracellular calcium, while only a transient calcium signal was observed in the activated eggs.

In accordance with the kinetics of Ca_i^{2+} release, the sequential activation of calpain and cathepsin was detected in the activated and hyperactivated eggs.

Monitoring of ROS levels in *Xenopus* eggs and oocytes treated with selective antioxidants (AOX) revealed that the specific inhibitors of the membrane NADPH-oxidase can potently modulate intracellular ROS levels and stabilize oocytes and eggs. Dose-dependent effects of 4-hydroxy-3-methoxyacetophenone (apocynin) were further investigated in detail.

The inhibitory effect of the MAPK pathway inhibitor U0126 on the timing of maturation, ovulation, and apoptotic initiation was revealed.

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 KI. (2017) Activity and distribution of senescence associated beta-galactosidase in aging oocytes and eggs. The 42nd FEBS Congress, September 13th, 2017, Jerusalem, Israel.

Tokmakov AA, Sato K. (2017) Effect of selective antioxidants on stability *Xenopus* oocytes and eggs. 88th Annual Meeting of the Zoological Society of Japan, September 21st, 2017, Toyama, Japan.

Ijiri TW, Shiba K, Sato H, Kawano N. (2018) Organizers of 第6_回生殖若手の会 筑波大学下田臨海実験センター February 28th - March 2nd, 2018, Shimoda, Japan.

Tokmakov AA, Sato KI, Stefanov VE. (2018) Postovulatory cell death: why eggs die by apoptosis in biological species with external fertilization. J Reprod Dev 64(1): 1-6.

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。