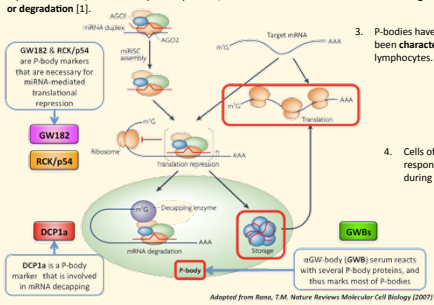
The role of mRNA processing bodies (P-bodies) in memory CD8⁺ T cells

Zinaida Tebaykina, Kate Choi, Lisa C. Osborne, Ninan Abraham, and Michael R. Gold
Department of Microbiology and Immunology, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z3

Life Sciences Institute

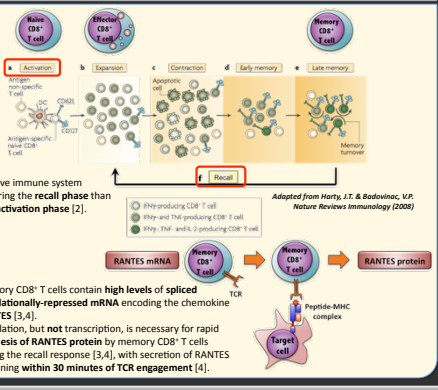
1 Introduction

1. mRNA processing bodies (**P-bodies**) are cytoplasmic aggregates that contain translationally-repressed mRNAs, as well as repressor proteins, and facilitate miRNA-mediated **mRNA storage or degradation** [1].



2. The partitioning of **mRNAs** between a **translationally-competent cytoplasmic pool** and a **translationally-repressed P-body pool** could be an important mechanism for dynamically controlling the synthesis of key immune response proteins.

3. P-bodies have **not** yet been **characterized** in lymphocytes.



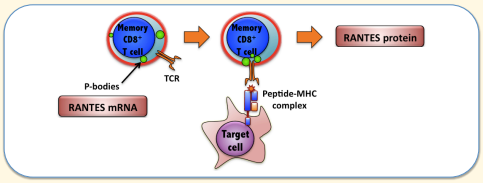
4. Cells of the adaptive immune system respond faster during the **recall phase** than during the initial **activation phase** [2].

5. Memory CD8⁺ T cells contain **high levels of spliced translationally-repressed mRNA** encoding the chemokine **RANTES** [3,4].

6. Translation, but **not** transcription, is necessary for rapid **synthesis of RANTES protein** by memory CD8⁺ T cells during the recall response [3,4], with secretion of RANTES beginning **within 30 minutes of TCR engagement** [4].

2 Hypotheses

Overall Hypothesis:
P-bodies are present in lymphocytes and store mRNAs that may be important for recall responses.



Hypothesis 1: T and B lymphocytes contain GW (P)-bodies and express the P-body markers GW182, RCK/p54, and DCP1a that are localized to discrete cytoplasmic foci.

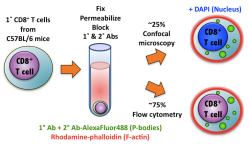
Hypothesis 2: Memory CD8⁺ T cells express elevated levels of P-body markers and contain more P-bodies than naïve CD8⁺ T cells.

Hypothesis 3: P-bodies of memory CD8⁺ T cells contain RANTES mRNA, and upon activation of a memory CD8⁺ T cell, P-bodies are recruited to the immune synapse where RANTES mRNA is translated.

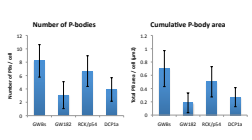
3 Result

Primary murine CD8⁺ T cells contain GWBs and express the P-body markers GW182, RCK/p54, and DCP1a that are localized to discrete cytoplasmic foci.

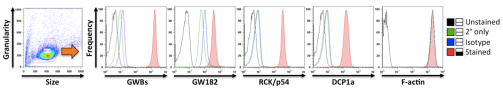
1. Dual analysis by flow cytometry and confocal microscopy was developed for both quantitative and qualitative analyses of P-bodies in a single sample of fixed lymphocytes.



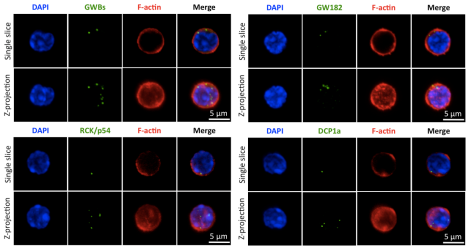
4. The number and cumulative area of P-bodies is different for each marker analyzed, suggesting that subsets of P-bodies with various protein compositions could exist.



2. Primary CD8⁺ T cells contain GWBs and express the P-body markers GW182, RCK/p54, and DCP1a.

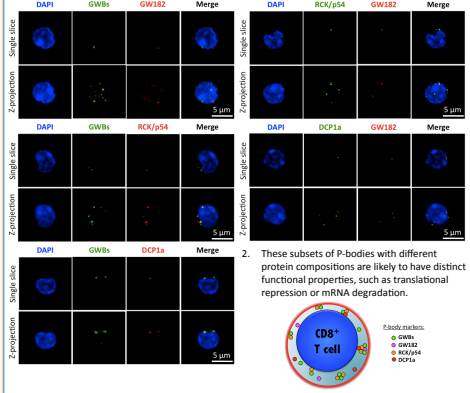


3. GWBs, GW182, RCK/p54, and DCP1a are localized to discrete cytoplasmic foci in primary CD8⁺ T cells.

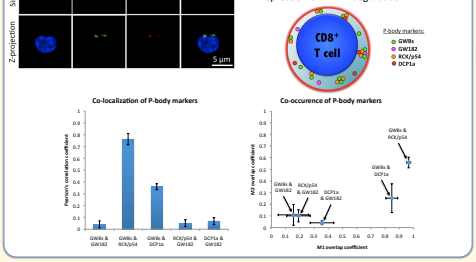


Primary murine CD8⁺ T cells contain subsets of P-bodies with different protein compositions and potentially distinct functional properties.

1. GWBs co-localize highly with RCK/p54 granules and partially with DCP1a granules, whereas GW182 rarely co-localizes with other P-body markers in primary murine CD8⁺ T cells.

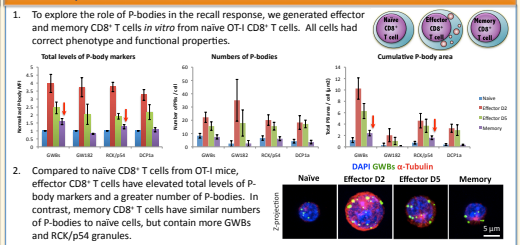


2. These subsets of P-bodies with different protein compositions are likely to have distinct functional properties, such as translational repression or mRNA degradation.

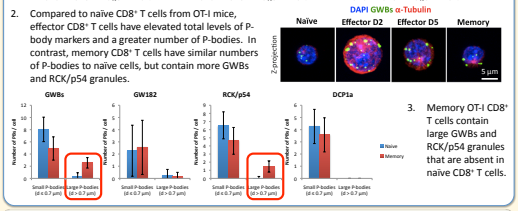


Memory CD8⁺ T cells contain large GWBs and RCK/p54 granules that are absent in naïve CD8⁺ T cells, but contain similar numbers of P-bodies.

1. To explore the role of P-bodies in the recall response, we generated effector and memory CD8⁺ T cells *in vitro* from naïve OT-I CD8⁺ T cells. All cells had correct phenotype and functional properties.

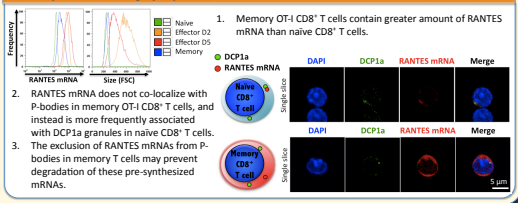


2. Compared to naïve CD8⁺ T cells from OT-I mice, effector CD8⁺ T cells have elevated total levels of P-body markers and a greater number of P-bodies. In contrast, memory CD8⁺ T cells have similar numbers of P-bodies to naïve cells, but contain more GWBs and RCK/p54 granules.



3. Memory OT-I CD8⁺ T cells contain large GWBs and RCK/p54 granules that are absent in naïve CD8⁺ T cells.

Memory CD8⁺ T cells highly express RANTES mRNA that is excluded from P-bodies.



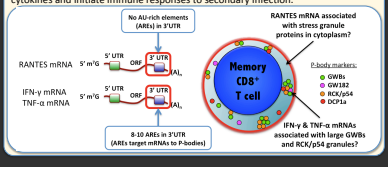
1. Memory OT-I CD8⁺ T cells contain greater amount of RANTES mRNA than naïve CD8⁺ T cells.
2. RANTES mRNA does not co-localize with P-bodies in memory OT-I CD8⁺ T cells, and instead is more frequently associated with DCP1a granules in naïve CD8⁺ T cells.
3. The exclusion of RANTES mRNAs from P-bodies in memory T cells may prevent degradation of these pre-synthesized mRNAs.

4 Conclusions

1. Dual analysis by flow cytometry and confocal microscopy is a useful technique for both quantitative and qualitative analyses of proteins and/or mRNAs in a single sample of fixed lymphocytes.
2. Murine and human T and B-cell lines, as well as primary murine T and B lymphocytes contain GWBs and express the P-body markers GW182, RCK/p54, and DCP1a, and that these proteins concentrate in discrete cytoplasmic granules. In mitotic T and B cells, most of GWBs and RCK/p54 granules dissociate, while GW182 and DCP1a granules remain intact.
3. P-bodies are heterogeneous in size and protein composition, raising the possibility that P-bodies with different protein components have distinct functional properties, such as translational repression or mRNA degradation.
4. Compared to naïve CD8⁺ T cells from OT-I mice, effector CD8⁺ T cells have elevated levels of P-body markers and a greater number of P-bodies. In contrast, memory CD8⁺ T cells have similar numbers of P-bodies as naïve cells, but contain substantially larger GWBs and RCK/p54 granules.
5. RANTES mRNA is excluded from P-bodies in memory CD8⁺ T cells, possibly protecting these mRNAs from degradation.
6. P-bodies accumulate in proximity of the immune synapse in naïve OT-I CD8⁺ T cells interacting with mature SIINFEKL-loaded BMDCs for 20', 1h, or 4h. In fact, P-bodies stay polarized towards the DC until the time of first division (28h). Similarly, P-bodies accumulate in proximity of the immune synapse in effector and memory OT-I CD8⁺ T cells.

5 Proposed Model

RANTES mRNA is stored in stress granules, while IFN-γ and TNF-α mRNAs are stored in P-bodies of memory CD8⁺ T cells, allowing memory cells to rapidly secrete cytokines and initiate immune responses to secondary infection.



6 Immediate Goals

1. Determine whether IFN-γ mRNA co-localizes with any of the P-body markers in memory CD8⁺ T cells.
2. Induce stress in memory CD8⁺ T cells and establish whether RANTES mRNA co-localizes with stress granules.

7 Acknowledgements

We would like to thank Dr. Marvin J. Fritzler from the University of Calgary for providing the human anti-GW6 serum.

This project is supported by a research grant from CIHR to M. R. G.

8 References

1. Rana, T.M. Illuminating the silence: understanding the structure and function of small RNAs. *Nat Rev Mol Cell Biol* **8**, 23-36 (2007).
2. Harty, J.T. & Badovinac, V.P. Shaping and reshaping CD8⁺ T-cell memory. *Nat Rev Immunol* **8**, 107-119 (2008).
3. Swanson, B.J., Murakami, M., Mitchell, T.C., Kappler, J. & Marrack, P. RANTES production by memory phenotype T cells is controlled by a posttranscriptional, TCR-dependent process. *Immunity* **17**, 605-615 (2002).
4. Walzer, T. *et al.* Immediate RANTES secretion by resting memory CD8 T cells following antigenic stimulation. *J Immunol* **170**, 1615-1619 (2003).