BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Pietro Fontana

eRA COMMONS USER NAME (credential, e.g., agency login): P_FONTANA

POSITION TITLE: Postdoctoral fellow

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Palermo	Bachelor	10/2010	Biology
University of Palermo	Master	10/2012	Molecular Biology
University of Palermo	Master	10/2014	Biotechnology
University of Oxford	DPhil	05/2019	Pathology

A. Personal Statement

During my DPhil I have specialised in learning techniques for protein biochemistry/enzymology and structural biology by X-Ray crystallography. With this expertise I identified the role of a new component of the DNA damage response, which we named Histone PARylation Factor 1 (HPF1). I demonstrated that HPF1 can lead to a novel post-translational modification of histone proteins named Ser-ADP-ribosylation, which consists of poly- or mono-ADP ribosylation specifically on serine residues. The discovery of Ser-ADP-ribosylation has already had a big impact in the field of ADP-ribosylation and become quickly a hot topic amongst researchers in this field. I subsequently identified ARH3 as the only human glycohydrolase able to remove this new modification. This new discovery again garnered a lot of attention within the field, and several papers have been published on ARH3 since. Moreover, I recently translated all my findings to a *Drosophila* model, which has led to the discovery of a new mechanism for Ser-ADP-ribosylation regulation, which is evolutionary conserved.

My long-term career goal is to become an independent group leader in immunology. Today's immunology research is mainly focused on cell biology and animal studies, which leaves a gap of information at the mechanistic level. For this reason, I wanted to extend my previous experience in biochemistry and complement it with newly acquired skills of structural biology. The Wu lab and Harvard Medical School are providing me with the necessary assistance and training in Cryo-EM, while I am able to contribute to the lab set of skills with my expertise in biochemistry and X-Ray crystallography. This combination of techniques is enabling me to characterize the NLRP1 inflammasome. With my work ethic and dedication to pursuing a research career, I am fully committed to maximizing the potential of my postdoctoral training as the current essential step for me on my pathway to becoming an independent researcher.

B. Positions and Honors

10/2012 Award academic merit-based university for Bachelor's degree.

10/2012 Special distinction awarded by the Master's Degree in Cellular e Molecular Biology committee "for the brilliant curriculum studiorum".

10/2014 Award academic merit-based university, Master's Degree in Cellular e Molecular Biology.

10/2014 Special distinction awarded by the Master's Degree in Biotechnology for industries and scientific research committee "for the significance of the scientific work conducted".

05/2019 Post-doctoral researcher in Hao Wu lab, Boston Children's Hospital/Harvard Medical School.

C. Contributions to Science

During my first master's degree I had the opportunity to contribute to a research project which ultimately showed that the amino acid tryptophan induces differentiation in *Stereptomuces coelicolor,* an actynomicete model largely studied for their antibiotic production.

Palazzotto E, Renzone G, **Fontana P**, Botta L, Scaloni A, Puglia AM, Gallo G. Tryptophan promotes morphological and physiological differentiation in Streptomyces coelicolor. ***Appl Microbiol Biotechnol***. 2015 Dec;99(23):10177-89.

My DPhil was focused on the biochemical and structural characterization of a novel component of the DNA damage response, which we named Histone PARylation Factor (HPF1). In the first paper, I identified HPF1 as a protein which regulates the activity of the key DNA repair enzyme poly-ADP-ribose polymerase 1 (PARP1) through an interaction with the catalytic domain of PARP1. PARP1 modifies itself and its substrates with the post- translational modification ADP-ribose. I showed that the HPF1-PARP1 interaction causes PARP1 to modify histone proteins rather than itself. In the second paper, I showed that HPF1 causes PARP1 to change amino acid target specificity from glutamate/aspartate residues to serine residues (Ser-ADP-ribosylation) on histones and other target proteins. To achieve this, I reconstituted the poly-ADP-ribosylation reaction *in vitro*, using just recombinant purified proteins.

Gibbs-Seymour I, **Fontana P**, Rack JG, Ahel I. HPF1/C4orf27 Is a PARP-1-Interacting Protein that Regulates PARP-1 ADP- Ribosylation Activity. ***Mol Cell***. 2016 May 5;62(3):432-42.

Bonfiglio JJ, **Fontana P**, Zhang Q, Colby T, Gibbs-Seymour I, Atanassov I, Bartlett E, Zaja R, Ahel I, Matic I.Serine ADP-Ribosylation Depends on HPF1. ***Mol Cell***. 2017 Mar 2;65(5):932-940.

Subsequently, I built on these findings and published the results in a first-author paper in *eLife*. By implementing a novel assay using a set of purified proteins, I managed to identify ARH3, a poorly characterized glycohydrolase, as the sole human enzyme responsible for removing the ADP-ribose modification from serine residues targeted by the HPF1-PARP-1 complex

Fontana P, Bonfiglio JJ, Palazzo L, Bartlett E, Matic I, Ahel I. Serine ADP-ribosylation reversal by the hydrolase ARH3. ***Elife***. 2017 Jun 26;6.

Using my unpublished structures, my colleagues reconstructed in vitro the mechanism that leads to Ser-ADP-ribosylation and identified a new unique enzymatic mechanism.

Suskiewicz MJ, Zobel F, Ogden TEH, **Fontana P**, Ariza A, Yang JC, Zhu K, Bracken L, Hawthorne WJ, Ahel D, Neuhas D, Ahel I. HPF1 completes the PARP active site for DNA damage-induced ADP-ribosylation. ***Nature***. 2020 Feb.

Finally, I extended my findings in a *Drosophila* model, leading to the discovery of a new mechanism for removal of serine-ADP-ribosylation that is evolutionarily conserved. The manuscript for this most recent work is complete and being prepared for submission to *Nature Structural and Molecular Biology*. I believe that my studies constitute a novel piece of work in the already complex epigenetic world.

Fontana P, Ariza A, Ahel I. Removal of Serine ADP-ribosylation in *Drosophila melanogaster*, a new mechanism. Manuscript in preparation for submission on ***NSMB***.

As a postdoc in the Hao Wu lab, my projects are focus on the structural and biochemical characterization of inflammasome, with particular focus on NLRP1.

Hollingsworth LR, David L, Li Y, Griswold AR, Ruan J, Sharif H, **Fontana P**, Orth-He EL, Fu TM, Bachovchin DA, Wu H. Mechanism of filament formation in UPA-promoted CARD8 and NLRP1 inflammasomes. ***Nature Communications***. 2021 Jan.

Hollingsworth LR, Sharif H, Griswold AR, **Fontana P**, Mintseris J, Dagbay KB, Paulo JA, Gygi SP, Bachovchin DA, Wu A. DPP9 sequesters the C terminus of NLRP1 to repress inflammasome activation. ***Nature***. 2021 Apr.

D. Additional Information: Research Support and/or Scholastic Performance

10/2012 ERSU scholarship for academic merit.

10/2014 Medical Research Council (MRC) DPhil scholarship, Oxford University.

10/2018 Medical Research Council (MRC) post-doctoral transition fellowship, Oxford University.

07/2020 Award CRI Irvington Postdoctoral Fellowship.