

BIOGRAPHICAL SKETCH

NAME		POSITION TITLE	
Emmanuele Crespan		Primo Ricercatore (Principal Investigator)	
EDUCATION/TRAINING			
INSTITUTION AND LOCATION	DEGREE (if applicable)	MM/YY	FIELD OF STUDY
University of Pavia	Master degree	07/2004	Biology
University of Pavia	PhD Degree	01/2008	Biochemistry and Molecular Biology
Russian Accademy of Science, Novosibirsk (Russia)	(Visiting student)	09/2006	Biochemistry and Molecular Biology
Institute of Molecular Genetics	(AIRC postdoc fellow)	2008-2011	Biochemistry and Molecular Biology
Institute of Veterinary Biochemistry and Molecular Biology – University of Zurich	(Visiting researcher)	07/2009-12/2009 01/2012-04/2012 03/2013-04/2013	Biochemistry and Molecular Biology

A. Personal Statement

I've been working all my career on the characterization of enzymes catalyzing reactions involving nucleic acid, complementing a biochemical approach with molecular biology and cellular biology ones. I'm involved in several long standing collaborations with medicinal chemistry groups focused on antiviral and antitumor development. I extensively worked on the characterization of repair DNA polymerases in cancer, in RNase H2 biochemical characterization and, more recently, on its interaction with DEAD-Box helicases. These enzymes are involved in the repair of the most abundant DNA lesions: genomic misincorporated ribonucleotides. I'm developing a tool for precise insertion of ribonucleotide into a desired sequence, which would allow to better study their repair. I'm extremely interested in the neurodegenerative disorder Cerebellar Ataxia, Neuropathy and Vestibular Areflexia Syndrome (CANVAS). Despite the disease-gene being known, CANVAS pathological mechanism was an enigma we partially solved very recently. I'm now focused on elucidation of such mechanism to elaborate possible treatments.

B. Positions and Honors (2019 – 2024)

Positions and Employment

YEAR	DESCRIPTION (Designation & Institution)
2011- 2023	Ricercatore III livello, Institute of Molecular Genetics, National Research Council, Pavia (Italy)
2024 - Present	Primo Ricercatore, Institute of Molecular Genetics, National Research Council, Pavia (Italy)

Honors

YEAR	DESCRIPTION (Designation & Institution)
2008	Cecilia Cioffrese Award for Cancer Rersearch, Fondazione Carlo Erba, Milano (Italy)
2014	Young Researcher Award, Department of Biomedical Sciences, National Research Council, Rome (Italy)

C. Contribution to Science

I worked my entire career in the field of DNA enzymology, studying DNA repair pathways that are related to different pathological conditions.

Main topics:

- Faithful 8-oxo-dG bypass by DNA Polymerase Lambda. Reactive oxygen species (ROS) can oxidise nucleotide bases by altering their chemical nature. As a result, oxidised nucleotide bases may pair unfaithfully or pose an obstacle to replication. The most common oxidative damage is the oxidation of guanine at position 8 (8-oxo-dG). This damaged base can pair efficiently with either the correct cytosine (C) or an adenine (A). Importantly, replicative polymerases preferentially misincorporate an A over an 8-oxo-dG. If not repaired, this mismatch leads to a G:C to A:T mutation. It is estimated that tens of thousands of 8-oxo-dGs are incorporated into the DNA of each replicating cell every day. These lesions are repaired by the Base Excision Repair (BER) mechanism, in which a glycosylase (OGG1) recognises the 8-oxo-dG:C pair and removes the damaged base. At this point, the resulting gap is filled by DNA polymerase beta or DNA polymerase delta. However, if an 8oxoG is not repaired and is present on the template strand during replication, the replicative polymerases will misincorporate an A, creating an A:8oxoG mismatch. This is recognised by the MUTYH glycosylase, which removes the A. At this point, a DNA polymerase must fill the gap, preferentially inserting a C to prevent the A:8-oxo-dG pairing from being restored. In 2007, we identified pol lambda as this enzyme and characterised this repair pathway [1-2].

1 - 8-oxo-guanine bypass by human DNA polymerases in the presence of auxiliary proteins. Maga G, Villani G, Crespan E, Wimmer U, Ferrari E, Bertocci B, Hübscher U. *Nature*. 2007 May 31;447(7144):606-8.

2 - Replication protein A and proliferating cell nuclear antigen coordinate DNA polymerase selection in 8-oxo-guanine repair. Maga G, Crespan E, Wimmer U, van Loon B, Amoroso A, Mondello C, Belgiovine C, Ferrari E, Locatelli G, Villani G, Hübscher U. *Proc Natl Acad Sci U S A*. 2008 Dec 30;105(52):20689-94.

- DNA Polymerase Lambda is involved in Microhomology-Mediated End Joining (MMEJ). As well as playing a key role in the faithful bypass of 8-oxo-dG, pol lambda is recruited by the Non Homologous End Joining (NHEJ) machinery during the repair of DNA double-strand breaks. In this mechanism, pol lambda fills short gaps formed within the broken ends. In addition to NHEJ, other alternative end joining pathways exist. These have emerged in pathological conditions where NHEJ is impaired, and are considered interesting targets for cancer treatment. One such pathway is MMEJ, which relies on resection, starting from the broken ends, of the 5'-3' strands until short homology sequences (up to 5 nts) are found. At this point, also the single stranded ends are resected up to the microhomology region, which is then annealed and elongated. We proposed that pol lambda plays a key role in this mechanism [3]. In particular, we showed that pol lambda is able to bridge and extend broken strands with microhomology ends by cooperating with the DNA damage sensor complex Rad9-rad1-hus1. These data have recently been confirmed in living cells, making pol lambda a valuable target for cancer therapy [4].

3 - Microhomology-mediated DNA strand annealing and elongation by human DNA polymerases λ and β on normal and repetitive DNA sequences. Crespan E, Czabany T, Maga G, Hübscher U. *Nucleic Acids Res*. 2012 Jul;40(12):5577-90.

4 - Pol λ promotes microhomology-mediated end-joining. Chandramouly G, Jamsen J, Borisonnik N, Tyagi M, Calbert ML, Tredinnick T, Ozdemir AY, Kent T, Demidova EV, Arora S, Wilson SH, Pomerantz RT. *Nat Struct Mol Biol*. 2023 Jan;30(1):107-114.

- Characterization of RNase H2. Ribonucleotides (rNMPs) misincorporated within the DNA by replicative and repair polymerases represent the most abundant lesions in the genome of all living organisms. The presence of rNMPs embedded in the DNA distorts the geometry of the double helix, impairing replication fork progression. Moreover, the 2'OH group makes rNMP prone to hydrolysis, leading to single strand breaks.

These lesions are removed through the RER pathway, which is initiated by RNase H2 that incises the phosphate-sugar backbone at the 5' of the DNA-embedded rNMPs. RNase H2 is an essential gene, extremely conserved throughout the living kingdoms, and the only enzyme showing this activity. RNase H2 is also able to resolve RNA/DNA hybrids by resecting the RNA strand. I've been focusing on RNase H2 activity for years, evaluating its ability to recognize rNMPs paired with damaged dNTPs, as well as characterising its ability to work on misincorporated rNMPs embedded in DNA secondary structures [5, 6]. More recently, I contributed to investigate the relation between RNase H2 and DDX3X. This is a DEAD-box helicase which, even if with lower efficiency, can incise a double strand DNA presenting embedded rNMPs. We now describe the physical and functional interaction among these enzymes, and the auxiliary role of DDX3X on RNase H2 activity [7].

5 - Impact of ribonucleotide incorporation by DNA polymerases β and λ on oxidative base excision repair. Crespan E, Furrer A, Rösinger M, Bertoletti F, Mentegari E, Chiapparini G, Imhof R, Ziegler N, Sturla SJ, Hübscher U, van Loon B, Maga G. Nat Commun. 2016 Feb 26;7:10805.

6 - High Flexibility of RNase H2 Catalytic Activity with Respect to Non-Canonical DNA Structures. Dede M, Napolitano S, Melati A, Pirota V, Maga G, Crespan E. Int J Mol Sci. 2021 May 14;22(10):5201.

7 - Synergistic action of human RNase H2 and the RNA helicase-nuclease DDX3X in processing R-loops. Secchi M, Garbelli A, Riva V, Deidda G, Santonicola C, Formica TM, Sabbioneda S, Crespan E, Maga G. Nucleic Acids Res. 2024 Oct 28;52(19):11641-11658.

- **Drug discovery.** Throughout my career, I have also collaborated with several medicinal chemistry groups in the effort to develop new drugs targeting different enzymes (DNA polymerases, helicases, protein and lipid kinases, viral proteases, and bacterial topoisomerases) involved in cancer progression, neurodegeneration, and viral and bacterial infections.

- **CANVAS.** In recent years, in collaboration with Andrea Cortese and Valentina Pirota, I've focused on characterising the molecular mechanism underlying the onset and progression of CANVAS [8, 9].

8 - Truncating Variants in RFC1 in Cerebellar Ataxia, Neuropathy, and Vestibular Areflexia Syndrome. Ronco R, Perini C, Currò R, Dominik N, Facchini S, Gennari A, Simone R, Stuart S, Nagy S, Vegezzi E, Quartesan I, El-Saddig A, Lavin T, Tucci A, Szymura A, Novis De Farias LE, Gary A, Delfeld M, Kandikatla P, Niu N, Tawde S, Shaw J, Polke J, Reilly MM, Wood NW, Crespan E, Gomez C, Chen JYH, Schmahmann JD, Gosal D, Houlden H, Das S, Cortese A. Neurology. 2023 Jan 31;100(5):e543-e554.

9 - Normal and pathogenic variation of RFC1 repeat expansions: implications for clinical diagnosis. Dominik N, Magri S, Currò R, Abati E, Facchini S, Corbetta M, Macpherson H, Di Bella D, Sarto E, Stevanovski I, Chintalaphani SR, Akcimen F, Manini A, Vegezzi E, Quartesan I, Montgomery KA, Pirota V, Crespan E, Perini C, Grupelli GP, Tomaselli PJ, Marques W; Genomics England Research Consortium; Shaw J, Polke J, Salsano E, Fenu S, Pareyson D, Pisciotto C, Tofaris GK, Nemeth AH, Ealing J, Radunovic A, Kearney S, Kumar KR, Vucic S, Kennerson M, Reilly MM, Houlden H, Deveson I, Tucci A, Taroni F, Cortese A. Brain. 2023 Dec 1;146(12):5060-5069.