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Titulo:

Role of human PolX DNA polymerases in the generation of chromosomal rearrangements by alternative-NHEJ

Resumen de la Memoria:

In mammalian cells, DNA double strand breaks (DSBs) are repaired mainly by non-homologous end joining (NHEJ). Recent evidence demonstrate that in the absence of classical NHEJ components there is an alternative NHEJ pathway (A-NHEJ) that shows nucleotide gain/loss and relies on microhomologies at repair junctions, and that seems to have an essential role in the generation of chromosomal translocations, a hallmark of cancer cells (Nussenzweig and Nussenzweig 2008). Although A-NHEJ is a robust pathway, very little is known about its molecular components. Analysis of the chromosomal junctions of the translocations produced by A-NHEJ shows junctional templated additions derived from the DNA ends involved in the two DSBs (Simsek and Jasin 2010). This is similar to that found at human chromosomal translocation junctions, and suggests the action of a DNA polymerase such as PolX DNA polymerases μ and/or λ. PolX DNA polymerases are required to fill-in gaps or to extend recessed 3¿-ends during the repair of DSBs that cannot be directly ligated. The action of these enzymes during NHEJ is related to their high capacity to bind and synthesize DNA from low stable template-primer structures (Wang et al 2001; Ruiz et al 2004; Nick McElhinny et al 2005). Supporting a role for mammalian PolX polymerases in the processing of DSBs in vivo by NHEJ, the analysis of Polμ- and Polλ-deficient mice revealed an impairment of the immunoglobulin rearrangements (Bertocci et al 2003 and 2005), which indicates an important role for these polymerases during V(D)J recombination. Equally, a role for Polμ in the repair of early-embryo RAG-induced DSBs has also been demonstrated (Gozalbo-Lopez et al 2008). The main goal of my research at present is to investigate how DNA ends generated after simultaneous DSBs are joined in chromosomal translocations occurring in eukaryotic cells, and the role of the human PolX DNA polymerases in this process. For this purpose, I will devise genetic assays in both yeast and mammalian cell models to generate in vivo two DSBs having non-complementary ends simultaneously, mimicking the circumstances that should occur in pre-cancerous cells. In these two systems, the role of the different PolX DNA polymerases during DSB repair process will be evaluated. This analysis will be carried out for either wild-type or mutant polymerases, each having differential biochemical and/or structural features, such as the absence of a BRCT domain to interact with other repair factors or an increased/decreased terminal transferase activity. I would like to remark that this generic post-doctoral position would be an ideal option to continue the research I have been doing in the last few years, as well as to keep collaborations with other laboratories. In this sense, I have already established collaborations with the laboratory of Dr. A. Aguilera (CABIMER, Seville), to devise the genetic assays in yeast, and with the laboratory of Dr. A. Ramiro (CNIO, Madrid), to analyze the role of Pol μ in the formation of Ig/myc translocations in spleen-derived B cells using our Pol μ KO mice model. Once completed the first stage of the project, a collaboration with the group of M. Jasin (MSKCC, NYC) will be established in order to translate the study to the mammalian system developed in her laboratory, which points toward the participation of Polμ in the generation of chromosomal translocations via A-NHEJ.

Resumen del Curriculum Vitae:

Bachelor degree in Biochemistry, Universidad de Granada (1998). PhD degree in Molecular Biology with cum laude distinction, Universidad Autónoma de Madrid (2004). Thesis title: " DNA polymerase mu (Polμ), an enzyme for DNA repair and variability ", director: Dr. Luis Blanco Dávila. The thesis work resulted in 4 publications as a first author: EMBO J 2000, Philos. Trans. Roy. Soc. London B 2001, Nucleic Acids Res. 2003 and 2004. Several collaborations during this time, including a short-term stay in the National Center for Biotechnology (A. Bernad¿s laboratory), also resulted in different contributions in other journals such as J. Mol. Biol., J. Biol. Chem. or European Journal of Immunol. Post-doctoral position in the Molecular Biology Department of the Andalusian Molecular Biology and Regenerative Medicine Centre (CABIMER) in Seville, under the supervision of Professor Andrés Aguilera. This work has resulted in 3 publications, 2 as a first author: Ruiz et al. (Mol. Cell. Biol., 2009), and Ruiz et al. (PLoS Genetics, 2011, in press). At the same time, I have taken part in some academic activities in the department of Genetics of the University of Seville, as assistant professor. To date, my scientific work generated up to 12 publications in recognized international scientific journals and has been communicated in 8 international meetings. Fellows: Graduate student fellowship "Formación de Personal Universitario", Ministerio de Educación y Ciencia (1999-2003). Postdoctoral Fellowship Juan de la Cierva, Ministerio de Educación y Ciencia (2005-2008). Assistant professor, Universidad de Sevilla (2009-2010). Postdoctoral Fellowship JAE-DOC2010, Consejo Superior de Investigaciones Cientificas (2010-2012). Altogether, my research involves an attempt at uncovering the interrelations between DNA repair and genomic instability in eukaryotic cells, and has had an notable impact on the scientific community as indicated by the publication of results in high visibility journals (530 citations), and its presentation in high quality international meetings. I carried out interdisciplinary research and knowledge on these topics, and expertise in molecular biology, biochemistry and yeast genetics. These work experiences provide me with an excellent background for developing the research line proposed.