Breve semblanza y bibliografía de Margarita Salas Falgueras

Pedro R. García Barreno
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Nacida, el 30 de noviembre de 1938, en Canero (Asturias), se doctoró en Bioquímica por la Universidad Complutense de Madrid. Ejerció como profesora de Genética Molecular en la Facultad de Químicas de la Universidad Complutense de Madrid (1968-1992) y profesora de investigación en el Centro de Biología Molecular Severo Ochoa (CSIC-UAM) (1974-2008).

Miembro de la European Molecular Biology Organization (EMBO, 1983), Real Academia de Ciencias Exactas, Físicas y Naturales (1988), Academia Europaea (1988), American Academy of Microbiology (1996), Academia Europea de Ciencias y Artes (1997), Academia Europea de Yuste (2004), American Academy of Arts and Sciences (2005) y U. S. National Academy of Sciences (2007).

Su intensa y larga carrera como investigadora ha sido reconocida con numerosas distinciones y premios, tales como: Severo Ochoa de Investigación de la Fundación Ferrer (1986), Carlos J. Finlay de UNESCO (1991), Rey Jaime I de Investigación (1994), Medalla del Principado de Asturias (1997), Premio a los Valores Humanos del Grupo Correo (1998), Premio de Investigación de la Comunidad de Madrid (1998), Premio México de Ciencia y Tecnología (1998), Medalla de la Sociedad Española de Bioquímica y Biología Molecular (1999), Premio Helena Rubinstein-UNESCO Women in Science (1999), Premio Nacional de Investigación Santiago Ramón y Cajal (1999), Española Universal por la Fundación Independiente (2000), Medalla de Oro de la Comunidad de Madrid (2002), Medalla de Honor de la Universidad Internacional Menéndez Pelayo (2003), Gran Cruz de la Orden Civil de Alfonso X el Sabio (2003), Premio Cristóbal Gabarrón de Ciencia e Investigación (2004), Medalla de Oro al Mérito en el Trabajo (2005), Medalla de Honor de la Universidad Complutense de Madrid (2005), Lección Conmemorativa Jiménez Díaz (2008), Premio

a «Toda una vida profesional» de la Fundación Mapfre (2009), Premio honorífico «Clara Campoamor» (2011), Premio a la Excelencia Química concedido por el Consejo General de Colegios Oficiales de Químicos de España (2014), Medalla Echegaray de la Real Academia de Ciencias Exactas, Físicas y Naturales (2016), Nature Mentoring Award in Science «for a life time achievement in mentoring» (2017).

Presidió la Fundación Severo Ochoa (desde 1997), el Real Patronato de la Biblioteca Nacional de España (2010-2015) y el Comité Ejecutivo de la Lección Conmemorativa Jiménez Díaz (desde 2016). Perteneció al Consejo Rector de la Agencia Estatal de Investigación (desde 2016). Fue nombrada doctora *honoris causa* por las Universidades de Oviedo (1996), Politécnica de Madrid (2000), Extremadura (2002), Murcia (2003), Cádiz (2004), Rey Juan Carlos de Madrid (2008), Málaga (2009), UNED (2011), UIMP (2011), Jaén (2012), Universidad Autónoma de Barcelona (2018) y Carlos III (2018). Fue nombrada marquesa de Canero por su valiosa entrega a la investigación científica sobre Biología Molecular (2008).

Su carrera científica está marcada por los siguientes hitos:

Tesis (Enzimología),
Prof. Alberto Sols, CSIC (Madrid)

La modificación por fosforilación es un paso del metabolismo de la glucosa. Su hallazgo fue describir que la actividad glucoquinasa que fosforila la glucosa era dependiente de la presencia de insulina. Relacionó ayuno/diabetes, insulina y fosforilación de glucosa. Salas M *et al.* (1963) *J Biol Chem* 238, 1175-1177.

Post-doc. (Biología Molecular),
Prof. Severo Ochoa, New York University

Severo Ochoa acababa de recibir el Premio Nobel de Fisiología o Medicina 1959 (compartido con el Prof. Arthur Kornberg) y seguía trabajando en el código genético: ADN → ARN → proteína (dogma de la Biología Molecular): La dirección de lectura del mensaje genético (mARN) para traducirse a proteínas es desde el extremo 5' al 3' (el extremo 5'C tiene un fosfato unido al carbón 5' de la ribosa. El extremo 3'C tiene un hidroxilo al carbón 3' de la ribosa). Salas M *et al.* (1965) *J Biol Chem* 240, 3988-3995. El triplete UAA (presente en mARN) es un codón de terminación para finalizar la síntesis de proteínas. Last JA *et al.* (1967) *PNAS* 57, 1062-1067. El trabajo desarrollado por Margarita Salas se ocupa de la dirección contraria a la del dogma de la Biología Molecu-

lar. Empezó trabajando en proteínas, luego en mARN y finalmente en ADN. Estos trabajos aparecen en algunos libros de texto.

Vuelta a España (junto a Eladio Viñuela), fago ϕ29

–ARN (transcripción): simultáneamente al descubrimiento de la estructura de la *E. coli* en Harvard, se produce en Madrid el de la estructura de la ARN polimerasa de *B.subtilis.* Avila J. *et al.* (1971), *Nature* 226, 1244-1245.

–ADN (replicación): se descubre que el ADN de ϕ29 tiene una proteína unida covalentemente a una de sus cadenas de ADN: Nuevo mecanismo de la replicación del ADN. Ortín J *et al.* (1971), *Nat New Biol* 234, 275-277.

–Nuevas tecnologías-Secuenciación de ADN: el genoma de un organismo vivo se secuencia por primera vez en España. Secuenciación del genoma (ADN) de ϕ29. Escarmis C. *et al.* (1981), *PNAS* 78, 1446-1450.

–ADN polimerasa de ϕ29-Purificación y caracterización. Blanco L. *et al.* (1984), *PNAS* 81, 5325-5329; Blanco L. *et al.* (1989), *J Biol Chem* 264, 8935-8940 (740 citaciones).

Biotecnología

La ADN polimerasa de ϕ29 es:

–Procesiva (capaz de añadir alrededor de 1000 nucleótidos por segundo al hidroxilo del extremo 3' del cebador).

–De las polimerasas conocidas, la que comete menos errores de lectura.

–Genera fragmentos de gran longitud.

–No necesita cambios de temperatura para los ciclos de amplificación.

–Requiere una mínima cantidad de muestra inicial para ser amplificada (ver Wikipedia: https://en.wikipedia.org/wiki/%CE%A629_DNA_polymerase).

En ese tiempo, Margarita Salas está colaborando con C. Richarson, colaborador a su vez de KB Mullis (Premio Nobel de Química 1993), por su invención de la PCR (*polymerase chain reaction*), hoy tan conocida con motivo de la identificación de portadores del virus SRAS-CoV2. Se decide patentar la polimerasa.

Patente de la polimerasa de ϕ29 (años 90), para hacer PCR mas exactas. Patente que ha sido la más beneficiosa para el CSIC.

Margarita Salas falleció en noviembre de 2019. En octubre de 2019 salió publicado su último trabajo relacionado con la polimerasa ϕ29 (Del Prado A *et al.* (2019) *Biomolecules* 9 (11), piiE648.

PRINCIPALES CONTRIBUCIONES CIENTÍFICAS

–Descubrimiento de una glucoquinasa específica para la fosforilación de glucosa en hígado de rata cuya síntesis depende de insulina.

–Determinación de que la lectura del mensaje genético transcurre en la dirección 5' a 3'.

–Descubrimiento de dos factores para la iniciación de la síntesis de proteínas en *Escherichia coli* encargadas de la unión del formilmetionil-tRNA a los ribosomas en presencia del triplete iniciador AUG.

–Demostración de la presencia de formilmetionina como iniciador de las proteínas codificadas por un mensajero policistrónico en un sistema de *E. coli*.

–Demostración de que el triplete sin sentido UAA da lugar a terminación de la cadena polipeptídica en un sistema de *E. coli*.

–Caracterización de las proteínas que forman parte de la estructura del bacteriófago ø29 y de la ruta morfogenética para su ensamblaje en la partícula viral.

–Demostración de que la proteína p4 del fago ø29 actúa como activador de la transcripción tardía del DNA viral mediante contactos directos entre la arginina 120 de la p4 y la región C-terminal de la subunidad α de la RNA polimerasa de *Bacillus subtilis*.

–Demostración de que la proteína p4 actúa como represor del promotor temprano A2c. En dicha represión se establecen los mismos contactos que en la activación del promotor A3.

–Demostración de que la activación o represión por la proteína p4 depende de la fuerza de las interacciones RNA polimerasa-promotor. Conversión del promotor tardío A3, que es activable por la proteína p4, en reprimible, y del promotor temprano A2c, que es reprimible por p4, en activable.

–Demostración de que la p6, que es una proteína tipo histona, coopera con la proteína p4 en la represión del promotor temprano A2c y en la activación del promotor tardío A3.

–Caracterización de una proteína unida covalentemente a los extremos 5' del DNA del bacteriófago ø29.

–Demostración de la existencia de un nuevo mecanismo de iniciación de la replicación por el cual la proteína terminal libre del bacteriófago ø29 actúa de iniciadora formando un enlace covalente con dAMP catalizado por la DNA polimerasa viral.

–Demostración de que la iniciación de la replicación del DNA de ø29 comienza en el segundo nucleotido desde el extremo 3' y propuesta de un

mecanismo de deslizamiento hacia atrás implicado en la fidelidad del proceso de iniciación.

–Demostración de la existencia de un paso de transición en la replicación del DNA de ø29 en el que la DNA polimerasa se disocia de la proteína terminal cuando ésta ha incorporado diez nucleótidos.

–Caracterización en la DNA polimerasa de ø29 de un dominio implicado en la actividad exonucleasa 3'→5' y un dominio implicado en las actividades de iniciación y polimerización. Demostración de la conservación de estos dominios en varias DNA polimerasas de organismos procarióticos y eucarióticos.

–Síntesis *in vitro* del DNA de ø29 utilizando la proteína terminal y la DNA polimerasa de ø29 como únicas proteínas.

–Amplificación *in vitro* del DNA de ø29 utilizando la proteína terminal, la DNA polimerasa, la proteína p6 que se une a los orígenes de replicación, y la proteína SSB de ø29. Demostración de que el DNA amplificado *in vitro* es infectivo.

–Las propiedades de la DNA polimerasa de ø29 (procesividad, desplazamiento de cadena y fidelidad) han dado lugar a su comercialización para amplificar DNA circular y DNA genómico lineal.

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Fecha de publicación: Octubre de 1991
Licenciado a: General Electric Healthcare

IN VITRO DNA SYNTHESIS REACTIONS USING PHI29 DNA POLYMERASE AND A DNA FRAGMENT ENCODING SAID POLYMERASE
Inventores: Luis Blanco, Antonio Bernad, Margarita Salas
Propietario: Consejo Superior de Investigaciones Científicas
Patente número: EP0527728
Fecha de publicación: Febrero de 1993
Licenciado a: General Electric Healthcare

REACCIONES DE SÍNTEIS IN VITRO QUE EMPLEAN DNA POLIMERASA DE PHI29 MODIFICADA Y UN FRAGMENTO DE DNA QUE CODIFICA DICHA DNA POLIMERASA
Inventores: Luis Blanco, Antonio Bernad, Margarita Salas
Propietario: Consejo Superior de Investigaciones Científicas

Patente número: ES2103741
Fecha de publicación: Octubre de 1997
Licenciado a: General Electric Healthcare

Inhibidor de la enzima uracil DNA glicosilasa y aplicaciones

Inventores: Gemma Serrano de las Heras, Alicia Bravo García, Margarita Salas Falgueras
Propietario: Consejo Superior de Investigaciones Científicas
Patente número: WO/2007/074200
Fecha de publicación: Julio de 2007

Método para la replicación, amplificación o secuenciación de un ADN molde

Inventores: Margarita Salas Falgueras, Miguel de Vega José, José M.ª Lázaro Bolós, Luis Blanco Dávila, Mario Mencía Caballero
Propietario: Consejo Superior de Investigaciones Científicas
Fecha de publicación: Julio de 2009
Licenciada a: X-Pol Biotech (Sygnis S. L. U.)

Inhibitor of the uracil-DNA glycosylase enzyme and use thereof

Inventores: Gemma Serrano de las Heras, Alicia Bravo García, Margarita Salas Falgueras
Propietario: Consejo Superior de Investigaciones Científicas
Patente número: US 8,129,336 B1
Fecha de publicación: Marzo de 2012

Utilización de señales de localización nuclear de proteinas de bacteriófagos como vehículo para transferencia de genes

Inventores: Margarita Salas Falgueras, Mario Mencía Caballero, Modesto Redrejo Rodríguez, Daniel Múñoz Espín, Isabel Holguera López
Propietario: Consejo Superior de Investigaciones Científicas
Fecha de publicación: Enero de 2014
Licenciada a: Sygnis S. L. U.

Quimera de ADN polimerasa del fago ø29

Inventores: Margarita Salas Falgueras, Miguel de Vega José, José M.ª Lázaro Bolós, Luis Blanco Dávila, Mario Mencía Caballero

Propietario: Consejo Superior de Investigaciones Científicas
Fecha de publicación: Mayo de 2011
Licensed to: X-Pol Biotech (Sygnis S. L. U.)

Phage phi29 DNA polymerase chimera
Inventores: Margarita Salas Falgueras, Miguel de Vega José, José M.ª Lázaro Bolós, Luis Blanco Dávila, Mario Mencía Caballero
Owner: Consejo Superior de Investigaciones Científicas
Patent number: US 8,404,808 B2; EP 2 450 436 B1
Date publication: March, 2013
Licensed to: X-Pol Biotech (Sygnis S. L. U.)

Método de amplificación de ADN basado en los orígenes de replicación del bacteriófago Ø29 y secuencias nucleotídicas asociadas
Inventores: Margarita Salas Falgueras, Mario Mencía Caballero, Miguel de Vega José, José M.ª Lázaro Bolós, Pablo Gella Montero
Propietario: Consejo Superior de Investigaciones Científicas
Fecha de publicación: Septiembre de 2012

Variantes de la DNA polimerasa de phi29 con termoactividad mejorada
Patent number: PCT/ES2016/070928
Inventores: Margarita Salas Falgueras, José M.ª Lázaro Bolós, Miguel de Vega José, Irene Rodríguez García
Propietario: Consejo Superior de Investigaciones Científicas
Fecha de presentación: Diciembre de 2015

Primer-independent DNA polymerase and their use for DNA synthesis
Patent number: P201731236
Inventores: Margarita Salas, Modesto Redrejo Rodríguez, Mart Krupovic, Patrick Forterre
Propietario: Consejo Superior de Investigaciones Científicas
Fecha de presentación: Octubre de 2017