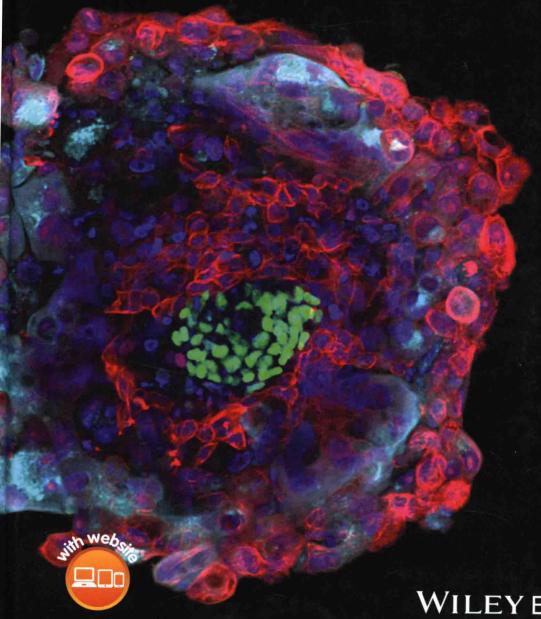
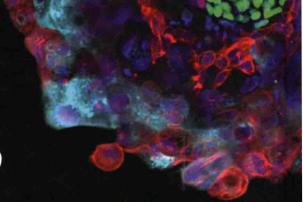
THE SCIENCE OF STEM CELLS

JONATHAN M. W. SLACK



WILEY Blackwell

THE SCIENCE OF STEM CELLS



Introduces all of the essential cell biology and developmental biology background for the study of stem cells

This book gives you all the important information you need to become a stem cell scientist. It covers the characterization of cells, genetic techniques for modifying cells and organisms, tissue culture technology, transplantation immunology, properties of pluripotent and tissue specific stem cells and, in particular, the relevant aspects of mammalian developmental biology. It dispels many misconceptions about stem cells—especially that they can be miracle cells that can cure all ills. The book puts emphasis on stem cell behavior in its biological context and on how to study it. Throughout, the approach is simple, direct, and logical, and evidence is given to support conclusions.

Stem cell biology has huge potential for advancing therapies for many distressing and recalcitrant diseases, and its potential will be realized most quickly when as many people as possible have a good grounding in the science of stem cells.

- · Content focused on the basic science underpinning stem cell biology
- Covers techniques of studying cell properties and cell lineage in vivo and in vitro
- Explains the basics of embryonic development and cell differentiation, as well as the essential cell biology processes of signaling, gene expression, and cell division
- Includes instructor resources such as further reading and figures for downloading
- Offers an online supplement summarizing current clinical applications of stem cells

Written by a prominent leader in the field, *The Science of Stem Cells* is an ideal course book for advanced undergraduates or graduate students studying stem cell biology, regenerative medicine, tissue engineering, and other topics of science and biology.

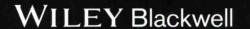
JONATHAN M. W. SLACK is a developmental biologist and author of five books and over 200 scientific papers. He is an emeritus professor at the University of Bath, UK, as well as the University of Minnesota, USA, where he was Director of the Stem Cell Institute from 2007–2013.



A companion website with additional resources is available at: www.wiley.com/go/slack/thescienceofstemcells

Cover Design: Wiley Cover Image: Gist F. Croft, Alessia Deglincerti, Ali Brivanlou, The Rockefeller University

www.wiley.com/wiley-blackwell







SLACK

websize 100

WILEY
Blackwell

The Science of Stem Cells

Jonathan M. W. Slack

WILEY Blackwell

This edition first published 2018 © 2018 John Wiley & Sons, Inc.

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, except as permitted by law. Advice on how to obtain permission to reuse material from this title is available at http://www.wiley.com/go/permissions.

The right of Jonathan M. W. Slack to be identified as the author of this work has been asserted in accordance with law.

Registered Offices

John Wiley & Sons, Inc., 111 River Street, Hoboken, NJ 07030, USA

Editorial Office

111 River Street, Hoboken, NJ 07030, USA

For details of our global editorial offices, customer services, and more information about Wiley products visit us at www.wiley.com.

Wiley also publishes its books in a variety of electronic formats and by print-on-demand. Some content that appears in standard print versions of this book may not be available in other formats.

Limit of Liability/Disclaimer of Warranty

The publisher and the authors make no representations or warranties with respect to the accuracy or completeness of the contents of this work and specifically disclaim all warranties; including without limitation any implied warranties of fitness for a particular purpose. This work is sold with the understanding that the publisher is not engaged in rendering professional services. The advice and strategies contained herein may not be suitable for every situation. In view of on-going research, equipment modifications, changes in governmental regulations, and the constant flow of information relating to the use of experimental reagents, equipment, and devices, the reader is urged to review and evaluate the information provided in the package insert or instructions for each chemical, piece of equipment, reagent, or device for, among other things, any changes in the instructions or indication of usage and for added warnings and precautions. The fact that an organization or website is referred to in this work as a citation and/or potential source of further information does not mean that the author or the publisher endorses the information the organization or website may provide or recommendations it may make. Further, readers should be aware that websites listed in this work may have changed or disappeared between when this works was written and when it is read. No warranty may be created or extended by any promotional statements for this work. Neither the publisher nor the author shall be liable for any damages arising here from.

Library of Congress Cataloguing-in-Publication Data

Names: Slack, J. M. W. (Jonathan Michael Wyndham), 1949- author.

Title: The science of stem cells / Jonathan M. W. Slack.

Description: Hoboken, NJ: Wiley, 2018. | Includes bibliographical references

and index. |

Identifiers: LCCN 2017028793 (print) | LCCN 2017030609 (ebook) | ISBN 9781119235231 (pdf) |

ISBN 9781119235255 (epub) | ISBN 9781119235156 (hardback)

Subjects: LCSH: Stem cells. | BISAC: SCIENCE / Life Sciences / Cytology.

Classification: LCC QH588.S83 (ebook) | LCC QH588.S83 .S575 2017 (print) |

DC 616.02/774-dc23

LC record available at https://lccn.loc.gov/2017028793

Cover Design: Wiley

Cover Image: Gist F. Croft, Alessia Deglincerti, Ali Brivanlou, The Rockefeller University

About the cover: Optical section through a day 12 human embryo which has developed in vitro. The epiblast (OCT4: green) has formed an amnion-like cavity. The trophectoderm has become cytotrophoblast (KERATIN 17: lilac) and multinucleated synciotiotrophoblast (hCG beta, acqua). DNA is stained red, showing cell nuclei. Image kindly supplied by Dr Ali H Brivanlou, Rockefeller University.

Set in 10/12pt Warnock by SPi Global, Pondicherry, India Printed in Singapore by C.O.S. Printers Pte Ltd

10 9 8 7 6 5 4 3 2 1

The Science of Stem Cells



Preface

This book originates from a widespread perception that many students studying stem cell biology, and even many junior workers in stem cell research labs, lack essential knowledge of the scientific underpinnings of the subject. This can lead to undesirable consequences, most notably the tendency for clinics to offer to patients "miracle cells" whose injection can cure all ills rather in the manner of a medieval elixir. Such unrealistic attitudes are also, unfortunately, highly prevalent among the general public. Excellent educational work is done by bodies such as the International Society for Stem Cell Research, which has an accurate patient website open to all. However, a correct perception among the public about the capabilities of stem cell therapy cannot be expected until the practitioners themselves have a clear idea of what sort of cells they are working with and what these cells can, and cannot, be expected to do. This book seeks to improve the situation by exploring the scientific basis of stem cell biology in a concise and accessible manner. It is designed to be suitable for all students studying stem cell biology at undergraduate or graduate school level.

The book deals with basic science and so does not cover the current clinical applications of stem cells. I considered that to include clinical material would make the book too long, lose focus, and cause it rapidly to become out of date. However, because of the inevitable demand for such information the book has an online supplement which summarizes briefly the state of play in each

clinical application of stem cells to date, and this may be found at www.wiley.com/go/ slack/thescienceofstemcells.

The "parent science" of stem cell biology is, to a large extent, developmental biology. Embryonic stem cells were discovered by developmental biologists, and the methods used for controlling their differentiation rely heavily on our knowledge of the normal mechanisms of regional specification and cell differentiation in the embryo. So developmental biology is necessarily an important part of this book. However, this is by no means just another textbook of developmental biology: it is primarily a book about stem cells and the concepts, technology, and experimental facts needed to understand them properly.

Because the focus is on the discussion of approaches and concepts the level of molecular detail has been kept fairly basic. Modern molecular life sciences are all very fact-heavy and comprehension can be obscured by too many facts. However, where readers require more detail on specific topics than is given here, it can always be acquired by reading recent review articles or key primary papers, a selection of which are cited at the end of each chapter. Although I have been sparing with molecular detail, I have listed where possible the major gene products that are indicative of the particular cell types which are of interest to stem cell biologists, and also some of the key physiological properties of these cell types. Familiarity with these criteria for cell identification is very important when assessing the results of experiments

involving the directed differentiation of pluripotent stem cells or the direct reprogramming of one cell type to another.

A further type of detail that is worth paying careful attention to is the difference between species. Most of the experimental work in this area has been done on the mouse, but stem cell biology is inevitably oriented toward developing eventual applications for human patients. I have been careful where possible to distinguish normal events in human and mouse, and also occasional results from other model organisms such as the zebrafish, so that students are not misled by an exclusive focus on the mouse. In keeping with normal convention the names of genes are italicized when they refer specifically to the gene or the RNA, and are in normal type when they refer to the protein.

The central message of the book is that there is nothing magic about stem cells. In fact, it turns out that stem cell behavior is more important than the stem cells themselves. Certain cell populations in the body may adopt a stem cell type of behavior under particular circumstances, depending on their developmental history and their environment. So being able to work with stem cells successfully means being aware of how cells behave in different contexts and understanding how to characterize and manipulate them properly.

In the long term, stem cell biology does have huge potential for generating novel therapies for many common and recalcitrant diseases, and this potential will be realized most easily when all students and practitioners can become real masters of the science of stem cells.

> Jonathan M. W. Slack Bath 2017

About the Companion Website

This book is accompanied by a companion website:

www.wiley.com/go/slack/thescienceofstemcells

which summarizes the current clinical applications of stem cells.



Contents

Preface xi

About the Companion Website xiii 1 What is a Stem Cell? 1 Stem Cell Markers 3 Label-Retention 4 The Niche 5 Asymmetric Division and Differentiated Progeny 6 Clonogenicity and Transplantation 6 In Vivo Lineage Labeling Conclusions 9 Further Reading 10 2 Characterizing Cells 13 Histological and Anatomical Methods 13 Histological Sections 13 Fixation 13 Sectioning 14 Staining 14 Electron Microscopy 15 Fluorescence Microscopy Wholemounts 17 Immunostaining 17 In Situ Hybridization 18 Other Methods 19 RNAseq 19 Laser Capture Microdissection 19 Flow Cytometry 20 Dividing Cells 21 The Cell Cycle 21 Studying Cell Turnover 24 Reporters for the Cell Cycle 26 Identification of Very Slow Cell Turnover 26 Classification of Cell Types by Proliferative Behavior 28 Cell Death 28 Further Reading 30

3 Genetic Modification and the Labeling of Cell Lineages 31 Introducing Genes to Cells 31 Transfection and Electroporation 31 Gene Delivery Viruses 33 Controlling Gene Expression 35 Tet System 35 Cre System 35 Inhibiting Gene Activity 37 CRISPR-Cas9 37 Transgenic Mice 38 Animal Procedures 38 Modification of Embryonic Stem Cells 40 Types of Transgenic Mice 41 Cell Lineage 42 Cell Lineage, Fate Maps, Clonal Analysis Use of CreER for Lineage Analysis 44 Retroviral Barcoding 46 Clonal Analysis in Humans Further Reading 47 4 Tissue Culture, Tissue Engineering and Grafting 49 Simple Tissue Culture 51 Media 51 Contamination 53 Growth in Culture 54 Cryopreservation and Banking 55 GMP Cultivation 56 Complex Tissue Culture 56 Induced Differentiation 56 Three Dimensional Cell Culture 57 Artificial Organs and Organoids 59 Grafting 60 The Immune System 61 T Cells 61 The Major Histocompatibility Complex 62 T and B Cell Responses Reactions to a Graft 64 Immunosuppressive Drugs 65 Animal Experiments Involving Grafting 66 Further Reading 67 5 Early Mouse and Human Development 69 Gametogenesis 70 Germ Cells 70 Mitosis and Meiosis 70 Primordial Germ Cells (PGCs) 72 Spermatogenesis 73 Oogenesis 73 Fertilization 76

Early Development 77 Preimplantation Phase Implantation Period – Mouse 80 Implantation Period – Human 82 Ethical and Legal Issues Concerning the Early Human Conceptus 85 Sex Determination 86 X-Inactivation 87 Imprinting 87 Cloning by Nuclear Transplantation (SCNT) 89 Further Reading 90

Pluripotent Stem Cells 93

Mouse Pluripotent Stem Cells Mouse Embryonic Stem Cells 93 Differentiation of Mouse ES cells Mouse iPS Cells Human Pluripotent Stem Cells 101 SCNT-Derived Embryonic Stem Cells Ethical Issues Concerning Human ES Cells Pluripotent Stem Cells from Postnatal Organisms 103 Applications of Pluripotent Stem Cells 104

Body Plan Formation 107

Further Reading 105

Embryological Concepts 107 Developmental Commitment 107 Embryonic Induction 109 Symmetry Breaking 110 Key Molecules Controlling Development 111 Genes Encoding Developmental Commitment 111 Inducing Factors 112 Wnt System 112 FGF System 113 Nodals and BMPs 114 Notch System 114 Hedgehog System 115 Growth Promoting Pathways 115 Retinoic Acid 115 Body Plan Formation 116 General Body Plan 116 Gastrulation 116 Embryo Folding 120

Organogenesis 125

Further Reading 123

Nervous System 125 The Brain 126 Regional Specification of the CNS 128 Rostrocaudal 128

Mediolateral 130 Dorsoventral 131 The Eye 131 The Neural Crest 132 Epidermis 134 Hair Follicles 135 Mammary Glands Somitogenesis 137 The Somite Oscillator and Gradient 138 Subdivision of the Somites 139 Myogenesis 140 The Kidney 140 Blood and Blood Vessels 142 Blood 142 **Blood Vessels** 143 The Heart 145 The Gut 146 Regional Specification of the Endoderm 148 The Intestine 149 The Pancreas 150 The Liver 150 Further Reading 151

Cell Differentiation and Growth 155

Organs, Tissues and Cell Types 155 **Epithelia** 156 Connective Tissues 156 Cell Differentiation 158 Regulation of Gene Activity 158 Lateral Inhibition 161 Asymmetrical Cell Division 162 Neurogenesis and Gliogenesis Neurons and Glia Neurogenesis 166 Gliogenesis 168 Postnatal Cell Division 169 Adult Neurogenesis 169 Neurospheres 171 Skeletal and Cardiac Muscle 171 Skeletal Muscle 171 Development of Skeletal Muscle 172

Muscle Satellite Cells 173

Cardiac Muscle 175

Endodermal Tissues 176

Cell Differentiation in the Pancreas

Cell Differentiation in the Intestine

Cell Differentiation in the Liver 179

Hepatocytes and Cholangiocytes

Liver Growth and Regeneration 181

Transdifferentiation and Direct Reprogramming of Cell Type Differentiation Protocols for Pluripotent Stem Cells 184 Further Reading 185

10 Stem Cells in the Body 189

The Intestinal Epithelium 189 Intestinal Stem Cells 191 In Vitro Culture 193 Clonality of Intestinal Crypts 193 The Epidermis 195 Hair Follicles 197 Cornea and Limbus 199 Mammary Glands 200 Mammary Stem Cells The Hematopoietic System 204

Analysis by Transplantation and in Vitro Culture 204 Hematopoiesis in the Steady State 207 The Hematopoietic Niche 209

Spermatogenesis 211 Further Reading

11 Regeneration, Wound Healing and Cancer 217

Planarian Regeneration 217 Neoblasts 218 Amphibian Limb Regeneration 220 The Regeneration Blastema 220 Pattern Formation in Regeneration 222 Mesenchymal Stem Cells Mammalian Wound Healing 225 Soft Tissue Wounds 225 Healing of Bone Fractures 225 Spinal Cord Injuries 227 Regeneration and Repair 228 Cancer 228 Genetic Heterogeneity of Cancer 230 Cancer Stem Cells 233

Index 239

Further Reading 236