

Chemistry 492 – General Biochemistry III

Portland State University, Spring 2011

General Information

Class meetings (**required**) – MWF 11:30 am – 12:35 pm in Neuberger 227

Text – Lehninger, *Principles of Biochemistry*, (5th edition), W.H. Freeman and Co.

Dr. Lehman's Office Hours – Mon & Fri 10–11 am; and by appointment

Best way to contact Dr. Lehman – by email! (niles@pdx.edu)

Information Over the Web

You can obtain much of the information needed throughout this course by accessing the following website:

http://www.chem.pdx.edu/%7Eniles/Lehman_Lab_at_PSU/Teaching.html

Here, you can see general information, gather up-to-date news, and download selected class notes and required readings as pdf files.

Prerequisites

The prerequisite for enrollment in Chem 492 is **completion of Chem 491 at PSU with a grade of C– or better**. I will assume you have taken a full year of organic chemistry in addition to Chem 490 and Chem 491. The textbook is required. You cannot use a text from previous year of Chem 492, in large part because this is the first year we will be using Lehninger, Fifth Edition.

Course Overview

Chem 492/592 is the third term in the chemistry majors' sequence in general biochemistry. As such, it is not a stand-alone course; it begins where Chem 491 left off. The theme of this third section of the biochemistry sequence will be the chemical basis of biological information storage and transfer. We will begin by reviewing the "central dogma of molecular biology", which depicts the basic flow of genetic information in biological systems, from nucleic acids to proteins. We will discuss the structure of nucleic acids, both in their "naked" form and complexed with proteins inside the cell. We will then go back and look at the metabolic pathways that give rise to nucleic acids in contemporary living systems. Then we will proceed to discuss sequentially the components of the central dogma: DNA replication, RNA transcription and reverse transcription, and protein translation. Although the overriding theme will be to become intimately familiar with the flow of biological information, we will strive to examine closely the chemical basis for each stage in this process. Consequently we will frequently and rapidly alternate between biological and chemical principles as if there were no fundamental distinction between the two. It will thus be to your great benefit to review chapters 1, 3, and (especially) 5 of the textbook before the end of the first week of class.

Grading

Grading will be done on a straight scale. Your grade will be determined by the total number of points that you accumulate on exams and on the assignments. There are a total of 500 points possible in the course. If you accumulate 425 points or greater (85%) you will receive an "A" or an "A–". If you accumulate 375 points or greater (75%) you will receive a "B–" or a "B" or a "B+". If you accumulate 325 points or greater (65%) you will receive a "C–" or a "C" or a "C+". If you accumulate 250–324 points you will receive a "D". If you accumulate fewer than 250 points you will receive a failing grade.

Plus and minus grade boundaries will be determined at the end of the course, but will generally be reserved for students that are clearly outside the statistical bubbles of most other A, B, and C students. Points can be obtained from the following:

- Midterm I (April 18th) – 100 points
- Midterm II (May 11th) – 100 points
- Reading Assignments (5 x 20 points each) – 100 points
- Final Exam (June 9th) – 200 points

You may take as many or as few of the midterm exams as you like. If you miss one or both midterms, the total points available on the exams that you do take will be scaled so that 500 total points are still possible. If you miss one midterm, the other midterm will be worth 150 points, and the final will be worth 250 points. If you miss both midterms, the final will be worth 400 points. You **MUST** take the final exam, no exceptions. The final will be graded more harshly so that students who take the midterms are not at a disadvantage. **(In fact it is clearly in your best interests to take all the tests!)**

Course Policies

Being a junior/senior level course (or a course for graduate students), I will treat you like adults and expect that you respond in a similar fashion and approach this course and the other students with respect. For many of you, this will be your final term at PSU, and by now you should have learned proper study habits and a high level of maturity with respect to learning. Some basic principles are:

- Cheating will not be tolerated.
- Plagiarism (passing off someone else's work as your own) will not be tolerated.
- Appropriate classroom behavior will be expected (*e.g.*, no talking during lectures).
- Late assignments (even by one minute) will not be accepted.

The midterm grading policy described above reflects this expectation to behave as independent, mature adults. If you feel, for whatever reason, you are not in a position to take a midterm exam, simply don't. Please do not feel the need to explain to me the reason why you missed the exam; I will trust your decision as a responsible adult. Whether you had a death or illness in the family, or whether the MAX line broke down on the way in from Beaverton, or whether you didn't study for the exam, or whether you simply overslept, the result is the same for me. The only requirement is that you take the final exam. You may, by at least one-week prior arrangement with me, take the final exam early, should you anticipate some conflict with the scheduled final exam time (Thursday, June 9, 2010 A.D. from 12:30 to 2:30 pm Pacific Daylight Time in same room as lecture). You may not take the final exam late (this time slot is the very last of all the possible time slots and I need to compile the grades later the same day. Moreover, the writing assignments (see below) will be expected (in hard copy on the lecture podium or by email in my "IN" box) by the beginning (*i.e.*, 11:30 am) of class on the day that they are due. Late assignments will be graded for helpful comments, but will receive a zero point score.

Writing Assignments

In this course we will place an emphasis on your ability to read and interpret the primary literature and communicate your learning through effective writing. There will be five writing assignments (see below) due on Mondays throughout the term (except the last, being due on the last Wednesday of class!). Each assignment will be worth 20 points possible. For each assignment, an article from the primary (*i.e.*, non-review) literature will be assigned that you will have to read carefully and critically. To convey your understanding of these articles, you will write and turn in a one-page paper that summarizes the **hypotheses tested**, **methods**, **findings**, and **future work needed** of each article. More details on the format of these one-page papers can be found below; see last page for a sample paper.

2011 Writing Assignments (papers will be available as pdf files at the course website, see above):

April 4 – Crick FHC & Watson JD (1954). *Proc. Roy. Soc. London A* **223**: 80–96. [DNA structure]

April 13 – Martick *et al.* (2008). *Nature* **454**: 899–902. [Hammerhead ribozymes]

May 2 – Changela *et al.* (2001). *Nature* **411**: 1077–1081. [DNA topoisomerase structure]

May 16 – Kamtekar *et al.* (2006). *Proc. Natl. Acad. Sci. USA* **103**: 10642–10647. [$\gamma\delta$ recombinase]

June 1 – Kruger *et al.* (1982). *Cell* **31**: 147–157. [*Tetrahymena* self-splicing RNA]

Grades will be assigned for each on a scale in 2-point increments (20 points = excellent job interpreting the paper and conveying your understanding; 16 or 18 points = good job, some minor flaws; 12 or 14 points = good job, at least one major flaw; 8 or 10 points = fair job, some misunderstandings regarding the paper and major flaws in interpreting it or presenting your interpretation; 2, 4, or 6 points = poor job, little demonstration that you understand the paper and/or failure to convey in writing your understanding; 0 points = assignment turned in late or not at all).

Examination Policies

The Midterm and Final exams in Chem 492 will be, unlike previous terms, 65 minutes and 2 hours long, respectively, taken during the regular class times. The exams will consist primarily of essay, short-answer, calculation, and matching questions. In general the questions themselves will not be too difficult, but you will be pressed for time in answering them. This will require you to know the subject matter thoroughly, and I recommend active studying as opposed to passive studying for these exams. I would not recommend coming to class late on exam days! You may not use any books, notes, or text-storing calculators during the exams. You should come to the exams with several pens or pencils and a calculator. Because the exams are torn apart during the grading process, you must write your name on every page of the exam to receive credit for that page.

Graduate Credit (Chem 592)

If you are a graduate student and taking this course for graduate credit, in addition to the above assignments, you will need to write a 10-page report on one topic chosen from the following list:

1. fluorescent dyes for nucleic-acid labeling
2. nuclear magnetic resonance (NMR) of nucleic acids
3. electron paramagnetic resonance (EPR) analysis of nucleic acids
4. infrared (IR) analysis of nucleic acids (1-dimensional and 2-dimensional)
5. electrospray (ESI) and matrix (MALDI) mass spectrometry (MS) analysis of nucleic acids
6. denaturing gradient gel electrophoresis (DGGE) analysis of nucleic acids
7. “locked” nucleic acids
8. homing endonucleases
9. RNA interference (RNAi)
10. riboswitches

This report must contain both background information on the topic as well as recent discoveries in the field. For the latter, the report must draw heavily on at least two, and preferably three recent (2009–2011) papers from *Biochemistry*, *Journal of Biological Chemistry*, *Chemistry & Biology*, *Nature*, *Science*, *Nature Structural Biology*, or *Cell*. No two students can choose the same topic, so discuss the issue with fellow graduate students in the class and with me early in the quarter. The reports can be handed in anytime during the term prior to the final exam (12:30 pm on June 9, 2010). The reports will be graded by triage: **excellent** (your grade will go up by 1/3 compared to your point total; *e.g.*, A– to A), **good** (your grade will remain the same as your point total), or **poor** (your grade will go down by 1/3 compared to your point total; *e.g.*, A– to B+).

2011 Lecture Schedule

Date	Reading	Topic
Mon, Mar 28	Ch. 1, pp. 22–26	Introduction; information flow in life
Wed, Mar 30	Ch. 8, pp. 271–274	Information theory; nucleotide structure
Fri, Apr 1	Ch. 8, pp. 275–287	Nucleotide structure; nucleic acids; base pairing
Mon, Apr 4***	Ch. 8, pp. 281; 287–289	DNA and RNA structure
Wed, Apr 6	Ch. 8, pp. 289–292; 296–298	Modified nucleotides; DNA damage; cofactors
Fri, Apr 8	Ch. 8, pp. 292–295	<i>DNA sequencing; automated nucleotide synthesis</i>
Mon, Apr 11	Ch. 22, pp. 882–888	Overview of nucleotide metabolism
Wed, Apr 13***	Ch. 22, pp. 888–891	Purines and pyrimidines
Fri, Apr 15	Ch. 3/9, pp. 89–90 317–319	<i>PCR; gel electrophoresis</i>
Mon, Apr 18	MIDTERM I {covering chapters 1 (part), 8 and 22 only}	
Wed, Apr 20	Ch. 25, pp. 975–989	Overview of DNA replication
Fri, Apr 22	Ch. 25, pp. 980–984	Mechanism of DNA polymerase
Mon, Apr 25	Ch. 24/25, pp. 958–960; 990	DNA topoisomerase & DNA ligase
Wed, Apr 27	Ch. 25, pp. 993–998	DNA repair: MMR, NER, BER
Fri, Apr 29	Ch. 25, pp. 998–1000	DNA repair: direct reversal
Mon, May 2***	Ch. 25, pp. 1003–1007	DNA recombination I
Wed, May 4	Ch. 25, pp. 1007–1016	DNA recombination II
Fri, May 6	Ch. 26, pp. 1021–1024	RNA and transcription overview
Mon, May 9	NO CLASS – MANFRED EIGEN'S BIRTHDAY	
Wed, May 11	MIDTERM II {covering pp. 958–960 and chapter 25 only}	
Fri, May 13	Ch. 26, pp. 1058–1059	<i>SELEX & in vitro evolution</i> (Guest lecture)
Mon, May 16***	Ch. 26, pp. 1033–1045	RNA processing
Wed, May 18	Ch. 26, pp. 1045–1050	RNA-directed catalysis (ribozymes)
Fri, May 20	Ch. 26, pp. 1050–1056	Reverse transcription; RDRPs
Mon, May 23	Ch. 27, pp. 1065–1074	The genetic code I
Wed, May 25	Ch. 27, pp. 1075–1079	The genetic code II; overview of translation
Fri, May 27	Ch. 27, pp. 1079–1087	Aminoacylation and aaRS enzymes
Mon, May 30	NO CLASS – MEMORIAL DAY	
Wed, June 1***	Ch. 27, pp. 1088–1091	Protein synthesis – initiation
Fri, June 3	Ch. 27, pp. 1091–1099	Protein synthesis – elongation/termination
Thu, June 9	FINAL EXAM {covering the entire course}	

{ *** denotes that a writing assignment is due that day **before** class begins: prior to 11:30 am *sharp*}

How to write one-page research article summaries

In this class I will expect you to learn how to read, process, summarize, and describe in writing original research papers from the primary scientific literature. Towards this end, I am requiring that each of you turn in (**on time**; see above) five one-page written summaries of important papers that have come out in the last half-century pertaining to nucleic-acid biochemistry.

The style I am hoping you will follow is that of the Perspectives section in *Science*, or the News & Views section in *Nature*, examples of both of which are to be found on the next few pages. These are roughly single page summaries of papers found later in the same issue of the journal. If you read these examples, you will find that the author first provides a context for the paper of interest. This is done with a few paragraphs of background about the general phenomenon and leads the reader to understand why the current paper is of interest. I don't expect several paragraphs of introduction, only one, but it should set the stage for the current paper. In fact, a good way to end the first paragraph is a sentence or two such as, "Now, Jones *et al.* have addressed this problem with a new experiment that examines whether pigs can indeed fly if fed enough turnips." In other words, it is in this first paragraph where you need to describe what hypothesis is being tested.

Once the stage has been set, the next paragraph or two in your summary should be devoted to explaining what the authors of the paper did. Here, by using a judicious sampling of the methods and results presented in the paper, you should relate the KEY experiments and the KEY findings. Don't simply reiterate every single last thing that was done, but you absolutely need to provide enough basic information so that your readers get a good sense of what approach the authors took. If I had a dime for every time I assigned a paper to review in which a structure was solved by X-ray crystallography ... and a student failed to mention that the authors even used X-ray crystallography ... I would be rich!!!

Your last paragraph must summarize the work succinctly and provide some indication of "what's next" in this line of research. Contrary to what you may think (now), no experiment has ever been done, or ever will be done, that solves a problem once and for all. You must think about what is missing from the current paper, what could be done better, and/or what the next logical experiment would be. There is no right or wrong answer to this, you may have to even use your imagination! But you WILL LOSE POINTS if you ignore this aspect of the assignment, trust me.

Here are a few important things to remember:

- Provide a catchy title for your review.
- Put yourself in the time frame of when the paper was published, not 2010. In other words, don't criticize Watson & Crick or Meselson & Stahl for not knowing modern biochemistry!!!
- You must reference the paper you are discussing. See the examples below. You need only do this once or twice, not every time you mention the work done. You may cite other papers too, but **if you do not cite the paper you are reviewing you will automatically lose 4 points**.
- Please use a common reference style for scientific papers. See the style I use above, or use the style of *Nature*, *Science*, *Cell*, *Biochemistry*, or some other journal. Pay attention to the details of how the citation is done: authors, year, title, volume, page numbers, *etc.* Be consistent.
- When a paper has more than one author, usually the work has been done in the laboratory of the LAST author. Also, the shorthand notation for these situations is the last name of the first author followed by "*et al.*" which is Latin for "and all the others". Note that "*et*" does not have a period after it, but "*al.*" always does even if it is not the last word in the sentence.

Improving Your Writing

1. There is no “proving” in science.

Under the scientific method, we formulate hypotheses and test them. The result is that we reject the hypothesis or fail to reject (provide support for) them.

2. References: Scott *et al.* (1977).

Scott *et al.* (*PNAS USA* 74: 193-197; 1977).

Scott JF, Eisenberg S, Bertsch LL, and Kornberg A (1977). A mechanism of duplex DNA replication revealed by enzymatic studies of phage ϕ X174: catalytic strand separation in advance of replication. *Proc. Natl. Acad. Sci USA* 74: 193-197.

3. The first sentence of a paragraph is the topic sentence.

All of the rest of the paragraph should explain, or at least flow from, the first sentence. Set the reader up for what he/she is about to read.

4. Draw the reader into your writing.

“In the study reported by Scott *et al.*, significant advances in our understanding of DNA replication were made by using a new *in vitro* enzyme system.”

5. Run-on sentences are bad. Semi-colons can help sometimes.

Sentences should convey one, or at most two, points. Two complete sentences that are tightly linked may be separated by a semi-colon; this should be used sparingly. Otherwise, it is best to start a new sentence.

6. Parallel sentence structure is required.

Bad: The bacterial enzymes studied were the rep protein and the DNA polymerase III holoenzyme and they also utilized the phage-induced cisA protein.

Good: The enzymes that were utilized were the bacterial rep and DNA polymerase holoenzyme, along with the phage-induced cisA protein.

7. Diction: *that* vs. *which* / *since* vs. *because* / *it's* vs. *its*

The bacterial enzyme **that** was responsible for strand separation was purified from *E. coli*, **which** is a bacterium **that** is commonly used in biochemistry. *E. coli* has been studied **since** the 1930's **because it's** easy to purify **its** proteins.

8. PRUFREAD YOOR PAPER BEEFORE HANGING IT'S IN!

9. Be sure to write *E. coli*, ¹⁴N, Smith *et al.* (1999), and use as few quotes as possible.

SPECTROSCOPY

Handedness in quick time

Patrick H. Vaccaro

The handedness of chiral molecules can be probed spectroscopically, but acquiring data can take hours, which is a problem for time-resolved studies. The latest method records such data in a flash.

Most daily encounters with chirality and chiral recognition go unnoticed, including such mundane events as putting on shoes, shaking hands with someone and admiring the helical patterns of seashells. But the concept that certain objects and interactions have an intrinsic 'handedness' permeates the entire fabric of science, and has a crucial role in diverse physical, chemical and biological processes. Of special importance for the molecular sciences are the different chemical and biochemical reactivities displayed by enantiomeric (mirror-image) compounds. Indeed, many drugs are single enantiomers; their mirror-image versions are often ineffective, or even harmful.

The development of tools to discriminate between enantiomers and to work out how chiral molecules interact is therefore an ongoing challenge. In particular, the unique signatures that can be obtained using optical probes that are based on chiral — circularly polarized — light (Fig. 1a) represent a long-standing, yet constantly evolving avenue of research¹. On page 310 of this issue, Rhee *et al.*² demonstrate a significant advance that promises to open new vistas in the realm of chiroptical spectroscopy, including the tantalizing possibility of interrogating chiro-specific phenomena with femtosecond temporal resolution (1 femtosecond is 10^{-15} seconds).

When electromagnetic radiation passes through a randomly oriented (isotropic) ensemble of non-chiral molecules — such as those in a gas, liquid or glass — its state of polarization, defined by the direction of oscillation of the electric-field vector, remains unaltered. This implies that the sample's frequency-dependent refractive index, n , and absorption index, κ , are independent of optical polarization. These parameters govern the speed and intensity of a wave propagating through the medium.

In contrast, light traversing a chiral medium experiences an intrinsic helical anisotropy, commonly referred to as optical activity, which causes right-circularly (R) and left-circularly (L) polarized light to undergo distinct retardation ($n_L \neq n_R$) and attenuation ($\kappa_L \neq \kappa_R$) processes¹. The differential retardation ($\Delta n = n_L - n_R$) leads to the phenomenon

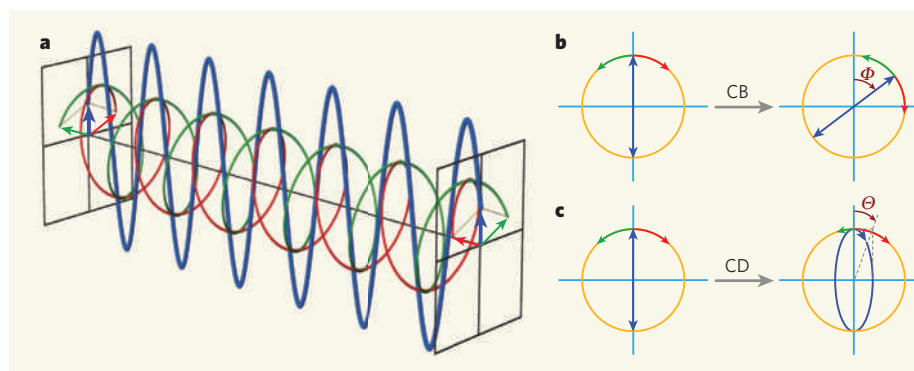


Figure 1 | Optical polarization and optical activity. **a**, The oscillating electric-field vector of linearly polarized electromagnetic radiation (blue) can be broken down into equal contributions of right-circular (red) and left-circular (green) polarized waves. **b**, Chiral media are optically active — they impose an intrinsic anisotropy on linearly polarized light. Here, the electric-field vector oscillates in a plane defined by the blue arrow. The phenomenon known as circular birefringence (CB) causes one component of circular polarization to propagate faster than the other, so that the plane of the electric-field vector rotates by an angle Φ . **c**, In circular dichroism (CD), one component of circular polarization is absorbed more strongly than the other, leading to net polarization ellipticity at an angle Θ . Rhee *et al.*² report a technique for acquiring information about the optical activity of chiral compounds on a femtosecond timescale, in principle opening the way to ultrafast time-resolved studies of chiro-specific chemical and biochemical processes.

of circular birefringence (CB, Fig. 1b), the observable effect of which is the rotation of linearly polarized light from its original angle; the differential attenuation ($\Delta\kappa = \kappa_L - \kappa_R$) causes circular dichroism (CD, Fig. 1c), in which R - and L -polarized light are absorbed unequally.

The two enantiomeric forms of a chiral compound display optical activities (Δn and $\Delta\kappa$) of equal magnitude, yet opposite sign, reflecting the chiro-specific nature of their interactions with oscillating electric and magnetic fields. The patterns obtained by recording CB or CD as a function of wavelength thus afford a spectral 'fingerprint' for a given enantiomer and its environment. The majority of such measurements rely on electronic excitations caused by radiation in the visible and ultraviolet regions of the spectrum. But analogous methods involving infrared light — which excites molecular vibrations — have also emerged as powerful probes of molecular structure and function^{1,3}. Rhee *et al.* build on the techniques of vibrational circular dichroism (VCD)^{4,5}, in which the unique chiroptical behaviour displayed by each vibrational feature of an

infrared absorption spectrum conveys information about the configuration and conformation of the molecule under investigation.

Unfortunately, the chiroptical response of an isotropic solution is exceedingly small, and so any viable probe of CD must be able to distinguish a minuscule chiral absorption signal from a substantially larger background of achiral absorption. For VCD (where the ratio of signal to background absorption is typically 10^{-5} – 10^{-6}), exquisitely sensitive detection schemes are needed to isolate the slight differences in sample absorption revealed by alternating bursts of R - and L -polarized infrared light. Even when the most efficient data-collection methods are used (such as Fourier-transform infrared spectroscopy⁴), hours of continuous averaging are often required to produce statistically meaningful VCD spectra, thus limiting the usefulness of the technique for time-resolved studies.

But Rhee *et al.*² have developed a strategy that permits both vibrational CB (known as vibrational optical rotatory dispersion, VORD) and VCD to be recorded without the need to

differentially discriminate minute signals. In their technique, a short infrared pulse is used to coherently excite vibrations in a dissolved chiral compound, thereby creating a macroscopic assembly of oscillating dipoles. This induced 'polarization' radiates the vibrational properties of the target molecules in the form of a signal known as a free-induction decay. This signal has chiro-specific information encoded in the amplitude and phase of its electric field — in the same way that the characteristic sound of a ringing bell reflects its size and shape.

So how is the weak chiroptical signal discriminated from the strong achiral background? The authors rely on the fact that linearly polarized light always develops another polarized component (perpendicular to the original direction of polarization) as it crosses a chiral medium (Fig. 1b, c). Using polarization analysers of exceptional quality aligned to detect only signals orthogonal to the impinging (linearly polarized) infrared beam, the authors were able to isolate the desired optical-activity response in their experiments.

To amplify and interpret the responses, Rhee *et al.* exploited the phenomenon of interference, in which the superposition of oscillating waves gives rise to distinctive patterns of new waves. They thus combined the free-induction decay from the chiral sample with an infrared reference pulse of much greater intensity. The coherently beating portion of the resultant pattern — the product of the weak signal field and the strong reference field — can be isolated and analysed using Fourier-transform manipulations⁶, yielding information about the sample.

Ultimately, the authors were able to ascertain a property of chiral molecules known as the complex susceptibility. This quantity consists of a part that is related to Δn and another part that is related to $\Delta \kappa$. In this way, VORD and VCD spectra can be acquired for each incident pulse of infrared radiation, with the timescale of each measurement essentially being given by the pulse duration (less than 100 femtoseconds).

Rhee *et al.* validated their approach by examining the enantiomers of a simple chiral molecule (limonene) dissolved in solution. They showed that the resulting spectra match those obtained by conventional VCD spectrometers and agree with theoretical VCD predictions. In principle, the techniques and concepts introduced in this work² should be applicable to a variety of chiroptical studies on systems in equilibrium, including those based on electronic (rather than vibrational) phenomena, provided that polarization analysers of sufficient quality are available for the targeted spectral region.

A time-resolved analysis of optical activity has yet to be performed, but the present results establish the basic principles required for studies in which changes in chirality induced by an abrupt pulse of 'pump' light can be monitored at subsequent points in time using a synchronously delayed infrared 'probe'. The resulting

arrays of VORD and VCD spectra should allow an unprecedented glimpse of the ultrafast structural and conformational changes that accompany chiro-specific chemical and biochemical transformations, such as those that occur during asymmetric catalysis or protein folding. Once the practical details are sorted out, the successful execution of time-resolved measurements will revolutionize our ability to interrogate chiral molecules and their interactions, in much the same way that the advent of high-speed photography overcame the limitations of visual perception. ■

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1. Barron, L. D. *Molecular Light Scattering and Optical Activity* 2nd edn (Cambridge Univ. Press, 2004).
2. Rhee, H. *et al.* *Nature* **458**, 310–313 (2009).
3. Barron, L. D. *et al.* *Mol. Phys.* **102**, 731–744 (2004).
4. Freedman, T. B., Cao, X., Dukor, R. K. & Nafie, L. A. *Chirality* **15**, 743–758 (2003).
5. Stephens, P. J., Devlin, F. J. & Pan, J.-J. *Chirality* **20**, 643–663 (2008).
6. Lepetit, L., Chériaux, G. & Joffe, M. *J. Opt. Soc. Am. B* **12**, 2467–2474 (1995).

CANCER

The nuances of therapy

Lee M. Ellis and David A. Reardon

Oncologists use drugs that limit a tumour's blood supply to prevent its growth. Although the initial effects of these drugs are beneficial to patients, new data suggest that their long-term effects warrant further study.

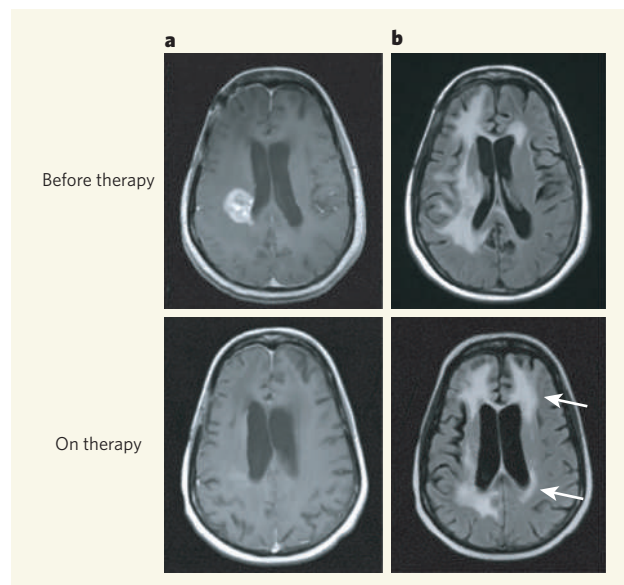
Angiogenesis — the formation of new blood vessels — is a hallmark of cancer, and allows tumour growth. Anti-angiogenic therapy offers great promise and is often used to treat cancer, either alone or in combination with chemotherapy. But, like all anticancer therapies, agents that inhibit tumour angiogenesis are prone to either intrinsic or acquired resistance. Pàez-Ribes *et al.*¹ and Ebos *et al.*² show in two preclinical (*in vitro* and animal) studies^{1,2} published in *Cancer Cell* that, depending on treatment conditions, anti-angiogenic therapy could theoretically increase the likelihood of tumour invasiveness and spread.

One protein with a central role in promoting angiogenesis is vascular endothelial growth factor (VEGF), and anti-VEGF agents are therefore commonly used to treat cancer.

Nonetheless, as highlighted by several studies, host and tumour responses to loss of VEGF-mediated signalling can be complex³.

In mice, deletion of a single copy of the *VEGF* gene causes embryonic death⁴, suggesting that it is essential for survival. Loss of this gene specifically in endothelial cells, which line blood vessels, increases the probability of angiogenesis-related disease conditions such as thrombosis, haemorrhage and fibrosis⁵. What's more, VEGF inhibition affects not only tumour vasculature but also healthy host tissues. When VEGF activity is impaired in tumour-free mice, for example, compensatory pathways are activated that in theory could augment tumour invasion and metastasis in patients with cancer. Ebos *et al.* have previously shown⁶ that anti-VEGF therapy increases levels of

Figure 1 | The MRI evidence. **a**, In agreement with the latest preclinical data^{1,2}, MRI scans from a patient with recurrent glioblastoma show that, after treatment with the VEGF-neutralizing antibody bevacizumab and the chemotherapeutic agent irinotecan, the macroscopic 'enhancing' tumour disappears, consistent with a complete response. **b**, However, microscopic tumour infiltration to other brain regions (arrows) is detectable in the patient after this therapy, using a different type of MRI that highlights brain inflammation and swelling¹¹.



D. A. REARDON

programmed antibodies in which antigen recognition is modified by the insertion of different ligands into the antibody binding site via a common reactive group (7). The beauty of the two-in-one molecule created by Bostrom *et al.* is its simplicity. For the first time, dual specificity has been engineered into a naturally occurring and stable antibody isotype that should pose no obstacles for manufacturing and that has been well validated for clinical use.

Two-in-one antibodies may replace combination therapies such as treatment of cancer with both bevacizumab and trastuzumab, which is currently in clinical trials. A practical limitation to this approach may be an inflexibility of dosing where optimal doses are discordant for the individual antigens targeted. A strong caveat comes from two recent studies that investigated the use of bevacizumab and chemotherapy in combination with either cetuximab (Erbix) or panitumumab (Vectibix) [antibodies that inhibit epidermal growth factor receptor (EGFR)] for treating metastatic colorectal cancer (8, 9). The studies showed that adding either of these antibodies to bevacizumab (plus chemotherapy) worsened clinical outcomes. These effects were unexpected because the antibody combinations had shown promise in the preclinical setting.

Two-in-one antibodies could also be used to target two nonoverlapping epitopes on the same antigen. Such antibodies would have a

greater potential for aggregating targets than classical antibodies. For combinations of either EGFR or HER2 monoclonal antibodies (10, 11), for example, such aggregation increases anti-tumor effects. The presence of two or more binding sites against distinct epitopes on a soluble antigen furthermore has the potential to increase binding avidity and *in vivo* potency (12).

The ability of antibodies to bind multiple antigens is, in itself, not a novel finding and has been described, for example, for the low-affinity binding of dissimilar peptides to distinct regions in a single antibody binding site (13). Indeed, by harboring multiple, spatially separated, binding sites in a single structure, antibodies may exploit a mechanism that has been recognized as a major source for multi-specificity of proteins (14, 15). The uniqueness of the work of Bostrom *et al.* is to show that promiscuous binding of antibodies is compatible with the high-affinity, pharmacologically relevant, binding of very different antigens. Promiscuous binding may even extend to natural immunity where it would represent a mechanism to maximally cover binding space by a given repertoire of antibodies. Cross-reactive antibodies, when isolated, are generally considered a nuisance and two-in-one antibodies may therefore have been overlooked. The increased availability of technologies for rapid and large-scale screen-

ing of antibody-antigen interactions should help identify promiscuous antibodies. The potential for high-affinity antibody binding of more than one antigen is intriguing and poses opportunities for future basic research and perhaps clinical development of antibody combination therapy.

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17. P. Parren is part of the management team of Genmab, a public company that develops human therapeutic antibodies including those against Her2 and VEGF. He is a named inventor on patents issued by Genmab. Amgen, Inc. is among Genmab's partners.

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MOLECULAR BIOLOGY

Dynamic DNA Methylation

Julie A. Law¹ and Steven E. Jacobsen^{1,2}

The silencing of gene expression through the methylation of cytosine nucleotide bases in DNA is observed in a wide variety of eukaryotic organisms. It occurs mainly at repetitive elements of genomes, and plays a critical role in silencing transposable elements (transposons). Its heritability is a key aspect of DNA methylation as a stable epigenetic mark of gene repression. However, two studies, by Teixeira *et al.* on page 1600 in this issue (1) and Slotkin *et al.* (2), show that DNA methylation and gene silencing can be much more dynamic than previously thought.

In the model plant *Arabidopsis thaliana*, three different methylation systems maintain

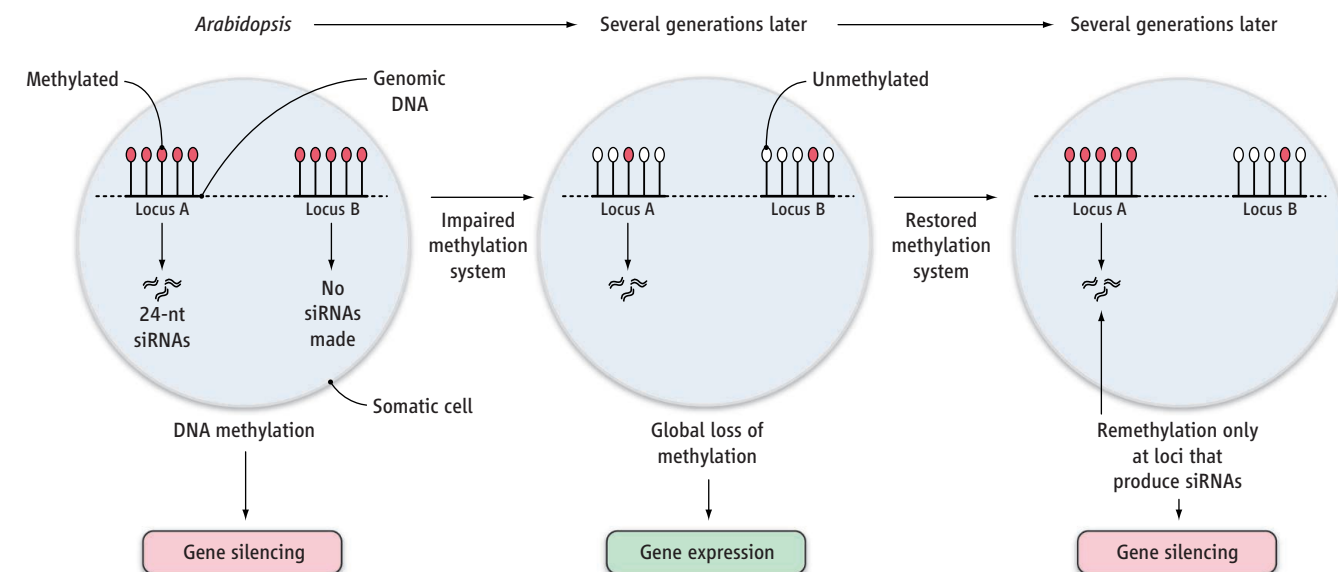
cytosine methylation in three different sequence contexts: CG [cytosine (C); guanine (G)], CHG [H is adenine (A), thymine (T), or cytosine (C)], and CHH (3). CG methylation is controlled by DNA METHYLTRANSFERASE 1 (MET1) and VARIANT IN METHYLATION 1 (VIM1) (4). The mammalian homolog of VIMI (UHRF1) recognizes hemimethylated CG DNA and facilitates its restoration to the fully methylated state (5, 6). Another critical factor is the chromatin-remodeling protein DECREASED DNA METHYLATION 1 (DDM1), whose mutation causes massive losses of methylation (7), and reactivates transposons (8). CHG methylation is maintained by the plant-specific CHROMOMETHYLASE 3 (CMT3), and KRYPTONITE (SUVH4), a histone protein methyltransferase. CMT3 binds to methylated histones (chromatin-associated proteins)

The methylation of DNA during plant development is a much more dynamic process than previously assumed.

and KRYPTONITE binds to methylated CHG sites, thereby creating a feedforward loop for maintaining CHG methylation (9). CHH methylation is controlled by a third DNA methyltransferase, DOMAINS REARRANGED METHYLTRANSFERASE 2 (DRM2). DRM2 is guided to its DNA targets by 24-nucleotide small interfering RNAs (siRNAs) in a pathway called RNA-directed DNA methylation (9, 10). In addition to maintaining CHH methylation, the RNA-directed DNA methylation pathway also controls the establishment of DNA methylation in all sequence contexts (11).

Although the details of these methylation systems are being quickly fleshed out, much less is known about the extent to which they are acting throughout plant development. Teixeira *et al.* show that some regions of the *Arabidopsis* genome can be efficiently

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Silenced, again. DNA methylation that is lost in previous generations (for example, through mutation of a gene required for methylation) can be restored in subsequent generations when a gene encoding the wild-type version of the protein is

reintroduced. However, remethylation is restricted to loci that produce siRNAs, and depends on the RNA-directed DNA methylation pathway. SiRNAs can thus selectively correct methylation defects to enforce silencing.

remethylated if methylation was lost in previous generations. Using *ddm1* mutants, which display a global reduction in DNA methylation, the authors investigated whether DNA methylation can be restored after a wild-type *DDM1* is reintroduced. Roughly half the sequences they examined regained methylation, thus reestablishing gene silencing. Complete remethylation was observed only after several generations, consistent with the multigenerational nature of transgene silencing known for plants.

The loci that became remethylated were characterized by the presence of high amounts of siRNAs, whereas loci that remained unmethylated lacked siRNAs (see the figure). Furthermore, reestablishing methylation required RNA-DEPENDENT RNA POLYMERASE 2, a key component of the RNA-directed DNA methylation pathway. Most siRNAs correspond to transposons and other highly repetitive DNA, which if expressed could lead to genome instability. Thus, the ability to specifically remethylate these sequences is likely beneficial in a multigenerational manner to reinforce silencing and to correct defects in methylation patterning that might otherwise lead to transposon activation.

In mammals, DNA methylation is dynamic during development, and examples include gene-specific imprinting as well as genome-wide changes in some cell types (12). *Arabidopsis* and other flowering plants imprint specific genes by selective demethylation of promoters in the endosperm (nutritive tissue in seeds of plants) (9), but whether methylation patterns are altered globally in

different plant tissues or cell types has been unclear. Slotkin *et al.* (2) report that the vegetative nucleus of *Arabidopsis* pollen cells shows a global loss of gene silencing, coupled with reactivation of transposon expression. Pollen contain three nuclei: the vegetative nucleus, which powers the cell; a sperm nucleus, which fertilizes the egg to form the zygote; and a second sperm nucleus, which fertilizes the central cell in the ovule to form the endosperm.

By comparing data from pollen with that of isolated sperm nuclei, Slotkin *et al.* (2) deduced that the vegetative nucleus was the location of transposon activation. Further, although new transposition events were detected in pollen, they were not inherited, again suggesting that transposon reactivation occurs in the vegetative nucleus, which does not contribute DNA to the zygote. Transposon reactivation was coupled with decreased expression of *DDM1*, and several genes that control RNA-directed DNA methylation, as well as reduced numbers of 24-nucleotide siRNAs. Interestingly however, a different class of transposon-related siRNAs (21 nucleotides in length) accumulates in pollen. The authors propose that these 21-nucleotide siRNAs, originating in the vegetative nucleus, may travel to the adjacent sperm cells to reinforce silencing, perhaps in a manner akin to that shown by Teixeira *et al.* for the remethylation of hypomethylated DNA in somatic tissue. Thus, only those transposons with the potential to be expressed (because they were expressed in the vegetative nucleus) would be targeted by siRNAs in sperm nuclei.

The results of Slotkin *et al.* raise the question of whether similar processes occur in the *Arabidopsis* female gametophyte—for instance, if loss of silencing in the central cell might cause reinforcement of silencing in the egg cell. There are also interesting parallels with the siRNA-mediated communication between nuclei seen in *Tetrahymena thermophila*, where small RNAs generated from the micronucleus target chromatin modifications (and eventually DNA deletion) to homologous genomic DNA sequences in the developing new macronucleus (13). In the future, it will be important to assess the extent to which the dynamic processes uncovered by these recent findings are utilized in other aspects of eukaryotic development.

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A Rare Catalytic Strategy Retained by Evolution

Article Review by Deborah Skaalrud

Ribosome is responsible for the protein synthesis in all living cells. The structure of ribosome has been made relatively apparent but the ribosomal catalysis remains controversial. Based on the structure of the enzyme-substrate complex, Nissen et al. in 2000 proposed a catalytic mechanism that involves with the base of A2486 functioning as a general base that facilitates the nucleophilic attack by the α -amino group of the A-site substrate. However, this mechanism cannot explain the later observations that mutations of the nucleotides involving the catalysis only have minimal effect on the reaction rate. Weinger et al.¹ explained this phenomenon by a possible catalytic mechanism that involves the P-site A76 2' OH with their new found evidence.

There were many models of the peptidyl transfer reaction and speculations made about the potential importance of the P-site A76 2' OH in catalysis; however, no consistent evidence was produced. Since the recent development of a peptidyl transfer assay, in which reaction rate is limited to proceed in a relevant to the physiological rate, Weinger et al. re-evaluated the role of P-site A76 2' OH in the peptidyl transfer reaction using full-size tRNAs with the 2' OH positions modified using dA and 2'-deoxy-2'-fluoro (fA) substitutions.

The authors recorded at least 10^6 -fold reduction in the reaction rate compared to the A76 tRNA, with no effect on the binding of the modified substrates. These observations indicated the A76 2' OH of the P-site tRNA in its full size is essential for donor activity and acts as the acceptor. The authors believe hydrogen bonding interactions between the 2'-OH and ribosomal bases are important for proper positioning of the P-site substrate and therefore causing the large rate effects produced by both dA76 and fA76. The authors also suggested a slight possibility that subtle conformational differences in the sugar pucker of A76 could have a substantial impact on the reaction if it proceeded exclusively from an O2'-linked peptidyl tRNA.

There are no functional groups in the vicinity of the ribosomal active site, except for the P-site A76 2' OH, that shows an impact on the rate of peptidyl transfer reaction; therefore, Weinger et al. concluded that the ribosome uses the P-site A76 2' OH for substrate-assisted catalysis. This conclusion is supported by the argument made by Sievers et al. in 2004 that substrate binding and positioning are sufficient to explain the observed rate enhancement. This mechanism is rarely found in modern protein enzyme catalysis but is found among group I self-splicing introns, which are ribozymes that use the O2' on the guanosine cofactor to coordinate a catalytic metal ion. Since the substitution of this 2' OH group vicinal to the O3' oxyanion nucleophile produced a similar magnitude of effects to the reaction rate, the authors believe that the P-site tRNA A76 2' OH may also coordinate a catalytic metal ion, such as Mg^{2+} or K^+ , for the peptidyl transferase reaction. Furthermore, metal ion coordination can reduce the pK_a of the 2' OH which can then participate in proton transfer by acting as a general acid and/or a general base. However, it can only be demonstrated if further experiments can produce an analysis of the peptidyl transferase center bound to relevant transition state inhibitors or substrate-product mimics through higher-resolution crystallography.

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