second polymorph. The quantity of solid is governed by the equilibrium constant for the dissolution process. Ultimately, the majority of solid will be the least soluble (most stable polymorphic form) in equilibrium with the drug in solution. The other polymorphic forms will be present in quantities dependent on their equilibrium constants. Quantification of the solubility for a metastable form can, therefore, result in considerable error depending on the time point selected for the assay of the solution phase.

A potentially more reliable method for the determination of solubility is the Noyes–Whitney template titration method[1]. Potentiometric analysis is achieved by titrating acid or base with the solid drug. Bjerrum Difference Plots can then be constructed from the titrations; these show the average number of protons bound in relation to pH, and provide approximate solubilities, which are then refined via iterative, least squares fit analysis[1]. This method has been applied successfully to the calculation of solubility for a series of polymorphs (Willson and coworkers, unpublished). A poster presentation can be viewed at the British Pharmaceutical Conference, 23–26 September 2001 (see http://www.rpsgb.org.uk). A commercial product (pSOL) is available from pION, Woburn, MA, USA (http://www.pION-inc.com)

Reference

1 Avdeef, A. *et al.* (2000) pH-metric solubility. 2: correlation between the acid–base titration and the saturation shake-flask solubility-pH methods. *Pharm. Res.* 17, 85–89

Richard J. Willson
GlaxoSmithKline
Harlow, Essex
UK CM19 5AW

Straightening out DNA replication – molecular combing ▼

In a recent issue of *Drug Discovery Today*[1] a review was published that discussed the emerging technique of molecular combing. This method enables replication to be viewed on a single-molecule basis and promises to provide insights into genome organization and cellular responses to DNA damage. The essence of this method is the attachment of linear molecules of DNA to a solid surface via their ends, followed by stretching – combing – and aligning by interaction of the DNA with a receding air–water interface. The result is molecules of DNA stretched out on a solid support that can be subjected to fluorescent *in situ* hybridization (FISH). When combined with pulse-chase labeling of DNA with bromodeoxyuridine (BrdU) or other nucleotide analogs, an unprecedented view of replication at the single-molecule level can be obtained.

As with all new techniques, standardization of the methods and verification of the uniformity of the results are work in progress. Also, the throughput is currently limited by manual inspection of the combed molecules, and automation will need to be developed if it is to be widely applied in drug discovery. However, even with these caveats, molecular combing can be applied in two broad areas – large scale genomic anatomy and DNA replication. Much of the description of genomic instability in cancer has relied on the analysis of population averages of molecules (from Southern blots, microarrays and PCR). Cytogenetics and sequencing analyze single molecules at the chromosomal and nucleotide levels, respectively. This leaves a gap of between a few and hundreds of kilobases. Combing can neatly fill this gap and will describe deletions, duplications and rearrangements currently missed by other methods.

Perhaps the most exciting application of this method is the description of ongoing replication at the single-molecule level in normal and tumor cells, with and without drug treatment. An effort should be made to describe, on a genome-wide scale, the location and firing of all origins of replication in both normal and tumor cell types using this method. Beyond this, there are several other questions:

- What are the consequences, at the molecular level, of DNA synthesis inhibitors on initiation, elongation and re-initiation?
- Do different inhibitors result in different consequences at the molecular level[2,3]?
- What are the responses of the replication machinery to DNA-damaging agents?

It has been known for decades that gaps are left opposite damage sites and that these are repaired by the extremely important and poorly understood daughter-strand gap-repair pathway[4]. The molecular structure of these gaps, the sites of initiating DNA synthesis downstream of the damage sites, and their repair, are not known. At a gross level, ionizing radiation induces the inhibition of both DNA synthesis (by inhibiting origin firing via a checkpoint pathway) and elongation (by direct blockage of the replication machinery). It has not been possible to describe this for most chemical agents using conventional methods, but here again molecular combing should prove useful. After inhibition of DNA synthesis by DNA damage, which origins of replication fire first? Is the temporal order of firing altered by damage? In cells that are defective in checkpoint signaling, is the origin of firing affected?

In summary, the advantage of molecular combing is the ability to analyze genome structure and replication molecule by molecule, something that has not been possible until now. As such, combing will probably join the battery of techniques available in the analysis of genomic instability in cancer.

1359-6446/01/$ – see front matter PII: S1359-6446(01)01984-5

References

1 Conti, C. *et al.* (2001) Targeting the molecular mechanism of DNA replication. *Drug Discov. Today* 6, 786–792

2 Linke, S.P. *et al.* (1996) A reversible, p53-dependent G0/G1 cell cycle arrest induced by ribonucleotide depletion in the absence of detectable DNA damage. *Genes Dev.* 10, 934–947

3 Marheineke, K. and Hyrien, O. (2001) Aphidicolin triggers a block to replication origin firing in *Xenopus* egg extracts. *J. Biol. Chem.* 276, 17092–17100

4 Friedberg, E.C. *et al.* (1995) *DNA Repair and Mutagenesis*. ASM Press, Washington DC

John R. Lamb
Program in Molecular Pharmacology
Clinical Research Division
Fred Hutchinson Cancer Research Center
Seattle
WA, USA