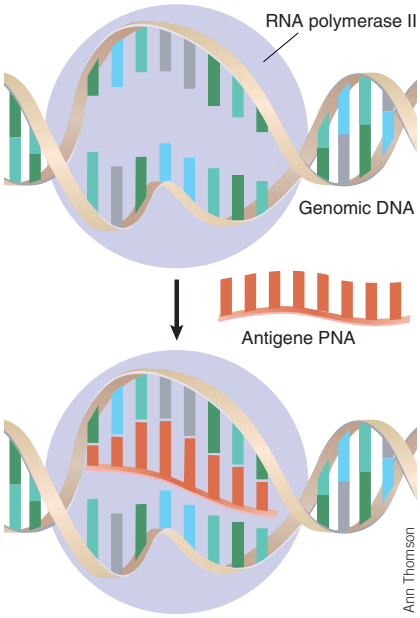

Figure 1 An antigene PNA is unable to bind to a complementary site within genomic DNA because of the base-paired structure. However, formation of the open complex with RNA polymerase before the start of transcription (top) allows binding of an antigene PNA to its target site, forming a hybrid PNA-DNA duplex that can inhibit transcription (bottom).

with increased expression of the cytoskeletal protein ezrin, for which expression levels are correlated with those of hPR[9]. The second interesting effect was that inhibition of hPR-B transcription also led to reduced expression of hPR-A, even though the transcription start site for the latter is 760 bases downstream and is not complementary to the anti-hPR-B PNA. This implies the existence of a feedback mechanism in which downregulation of hPR-B leads to downregulation of hPR-A.

The second paper from Corey's laboratory describes the use of duplex RNA to interfere with transcription. The RNA duplexes, reminiscent of siRNA, targeted regions consisting of 19 nucleotides in the vicinity of transcription start sites and inhibited transcription at low-nanomolar concentrations. In addition to hPR, specific antigene effects were observed for three other genes when targeted by RNA duplexes having the appropriate sequence. Sequence selectivity was very good, with two mismatches being sufficient to preclude knockdown.

In contrast to the antigene PNAs, which most likely exert their effects through formation of PNA-DNA duplexes with the open complex, the inhibition mechanism for the antigene RNA duplexes is less clear. To form an analogous RNA-DNA duplex, the antigene RNA would have to first shed its complementary strand and then hybridize to the open complex. Although this is possible, the authors acknowledge that a mechanism involving protein-mediated binding to mRNA transcripts to form a complex that then interferes with transcription might also occur. One intriguing result that is difficult to reconcile with the simple hybridization mechanism is the finding that shifting the target site by one base eliminates the inhibitory effect, whereas shifting it one base further restores it. It will be interesting to see whether analogous experiments with the antigene PNAs show similar target-site dependence or whether the PNA can be 'scanned' through this region without loss of function.

Antigene PNA oligomers and RNA duplexes are promising tools for specific gene knockdown at the level of transcription. Although such agents will not allow analysis of downstream control processes such as alternative splicing, other strategies can be used in such cases. It will also be important to look more broadly for off-target effects, possibly through the use of gene chips. Nevertheless, strategies based on the ability to target the open complex should efficiently and selectively inhibit transcription.

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Watching proteases in action

Klaudia Brix & Silvia Jordans

Activity-based probes can be used for monitoring enzyme activity based on their covalent reactions with active-site residues. A quenched activity-based probe has now been developed that becomes fluorescent only after labeling active proteases. The specificity of the fluorescent signal and cell permeability of the small molecule make this probe effective for monitoring protease activity in living cells.

Genes encoding proteases within any genome are easily identified, and three-dimensional structures of many proteases have been solved. Most proteolytic cleavage mechanisms and protease substrate specificities have been established (the latter in some detail)[1]. We are also able to block proteolytic activities with potent inhibitors. Transgenic approaches have been used for targeted deletions of protease- or protease inhibitor-encoding genes, providing important insights into the biological significance of proteases[2]. Given that we know a fair amount about these enzymes, why do we still not know when and where proteases cleave their natural substrates? The answer is simply that watching proteases in action is not a trivial task. In this issue of *Nature Chemical Biology*, Matthew Bogyo and colleagues describe elegantly how to use chemistry for the design of quenched activity-based probes (qABPs) that can be applied to visualize the dynamics of active proteases[3]. The new probes enable imaging proteases in real time and at the point of their action. The next generation of these probes may prove suitable for whole-body imaging of proteases for biomedical diagnostics.

Why should we be interested in exploring the labyrinth of proteolysis *in vivo* and in such detail? Cells use proteases for many critical cellular functions, including protein degradation, prohormone processing[4], antigen presentation and induction of programmed cell death. Proteolysis is rapid and

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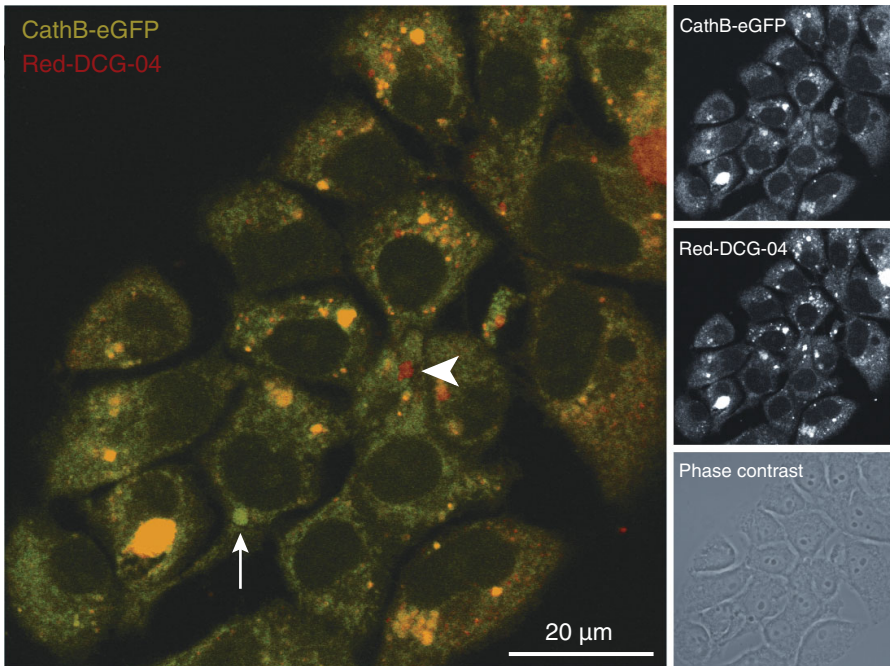

Figure 1 Comparison of techniques used to visualize proteases. GFP tagging allows the analysis of protease expression and trafficking in living cells. ABPs, such as DCG-04 or the newly designed GB111 and GB117 (ref. 3), depict only the active forms of cysteine proteases. Note that protease expression (green) is visible within ER, Golgi and secretory vesicles (arrow), whereas active proteases are present in endolysosomal compartments (yellow). Red vesicles are indicative of active cysteine protease–containing compartments that do not contain enhanced GFP-tagged cathepsin B (arrowhead). CathB-eGFP, enhanced GFP-tagged cathepsin B. Red-DCG-04, red fluorophore on DCG-04.

cleavage is irreversible. Proteases that cleave at the wrong position, attack the wrong substrate or otherwise function incorrectly may cause severe diseases[5]. Diseases involving improperly functioning proteases include Alzheimer disease, arthritis, cancer and osteoporosis, as well as infectious diseases such as AIDS, Ebola, malaria and sleeping sickness[6]. Therefore, to understand the physiology and to better explain the onset and progression of diseases, we need to know which protease cleaves exactly where, when and under what biochemical conditions.

Many approaches have been developed to visualize protease activities. Classical enzyme cytochemistry has been used for light and electron microscopic detection of proteolytic activities, the great advantages of which are selectivity and high resolution. However, because fixation steps are unavoidable, enzyme cytochemistry is not suitable for monitoring proteases *in vivo*[7]. In living cells, proteases have been visualized through fluorescent protein tagging. The fluorescent protein tags do not seem to hinder trafficking or proteolytic activities of the enzymes (**Fig. 1**). Using green fluorescent protein (GFP) to illuminate proteases in living cells has the great advantage of high spatial and temporal resolution[8]. However, GFP tagging has a number of disadvantages, including the requirement for cloning and transfection steps, and GFP visualizes both inactive preproteases and active proteases.

ABPs are small, diffusible molecules that can even be used for an indirect readout of activity levels, because they exclusively label active enzymes. Many different ABPs have been developed that are suitable for diverse experimental settings. For instance, they may carry a variety of reporters, including biotin or fluorophores. Hence, these probes can be used for labeling cell lysates, but they can also be effective in living cells and even whole organisms[9]. ABPs are instrumental for assessing the complexity of proteolytic activities in proteome-wide approaches[10]. As such, ABPs have recently been used to monitor protease activities in mouse cancer models.

What is so special about the newly designed qABPs? It is the quenching. Tagged ABPs are constitutively fluorescent. As a result, they cause a high nonspecific fluorescent background and a low signal-to-noise ratio if used in living cells. In contrast, qABPs contain a fluorescence donor and acceptor and so are not fluorescent before reacting with an enzyme target. When qABPs encounter their protease target, the fluorescence acceptor is released and the probe becomes fluorescent. Through the combination of cell permeability and covalent binding to the active site cleft, qABPs sensitively label active cysteine proteases in living cells[3]. The probe-labeled proteases can then be assessed through either fluorescence microscopy or classical biochemistry. Compared with the use of standard ABPs, this strategy has the great advantage of igniting the active protease, with improved signal-to-noise ratios resulting in enhanced spatial and temporal resolution. In short, qABPs switch a light on cysteine proteases. Thus, they make high-contrast imaging of proteolysis as simple and easy as viewing fireflies at night.

Bogyo and colleagues[3] targeted the proteases cathepsins B and L using their qABPs. These enzymes belong to the family of papain-like cysteine proteases. Classically, cathepsins were referred to as lysosomal enzymes and believed to be primarily, if not exclusively, catabolic in function. Now, it is well accepted that they are also able to cleave substrates at unexpected locations such as in the extracellular space. Hence, cathepsins have been suspected of facilitating tumor cell invasion[11]. However, it is important to know the exact location of protease activity to determine whether proteases of tumor or stromal cells are the key figures in cancer biology. Therefore, these new probes will allow more precise future investigation into the regulation of tumor progression.

Although the newly reported qABPs will certainly help in answering questions about the regulation and fine-tuning of biologically relevant actions for this subset of cysteine proteases, the development of additional qABPs with different specificities will be helpful in tackling a wide variety of other proteases. Without any toxicity information at hand, it is difficult to judge the suitability of qABPs for use in animals. However, future studies will reveal whether these or closely related probes will enable whole-body protease imaging.

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