are difficult to crystallize, such as large complexes and proteins embedded in membranes. Recently, a structure was determined with a FEL using nanocrystals prepared by overexpressing a protein in insect cells[7] (Fig. 1). This method of preparation seems to be applicable to many proteins, and could save years that would otherwise be spent crystallizing proteins using conventional methods.

FELs also overcome one of the main obstacles in crystallography: that proteins are often damaged by conventional X-ray sources. X-ray pulses from FELs are extremely intense and so completely destroy molecules and crystals. But because the pulses have only femtosecond duration (1 femtosecond is 10^{-15} seconds), diffraction patterns can be detected before the molecules are destroyed[8]. This overcomes the size limit for crystals, as noted earlier. It also allows damage-free structures to be determined from radiation-sensitive crystals. This is especially important for proteins that contain metal centres, which tend to undergo X-ray-induced chemical reduction.

Biomolecules are dynamic, but most crystal structures provide only a static picture of such molecules in one state. By contrast, time-resolved femtosecond crystallography using FELs allows researchers to make 'molecular movies' — a series of snapshots — of biomolecules in action. For proteins whose reactions can be triggered by light, X-ray pulses fired at different times after a light trigger enable the structures of different reaction intermediates to be obtained[9].

Not all protein reactions are light driven, however. Methods are therefore being developed in which rapid mixing of protein nanocrystals with a solution of the protein's substrate triggers a reaction; X-ray pulses are then fired at the sample at different time intervals after mixing. This should enable all the steps of drug transport through a receptor to be visualized, for example.

The current main limitation of structural biology research with FELs is access to beam time at the two sources in the United States and Japan. But, with the opening of the European FEL and the Swiss FEL in 2015 or 2016, available beam time will increase significantly. Furthermore, the European FEL will allow up to 10,000 images to be collected per second, so that a full data set can be acquired in 5 minutes, rather than the 3 hours required at present.

It is the dream of structural biologists to determine atomic structures from the X-ray diffraction of single molecules, but this is not yet within our grasp. To reach this goal major challenges have to be met: the flux of X-ray photons from FELs must be increased by at least 1,000-fold to detect the weak diffraction of individual biomolecules at atomic resolution. In addition, the duration of pulses may have to be shortened to less than a femtosecond, to allow for diffraction before destruction of single molecules. ■

Petra Fromme *is in the Department of Chemistry and Biochemistry, Arizona State University, Tempe, Arizona 85287-1604, USA.*
e-mail: pfromme@asu.edu

1. Riekel, C., Burghammer, M. & Schertler, G. *Curr. Opin. Struct. Biol.* **15,** 556–562 (2005).
2. Smith, J. L., Fischetti, R. F. & Yamamoto, M. *Curr. Opin. Struct. Biol.* **22,** 602–612 (2012).
3. www.nobelprize.org/nobel_prizes/chemistry/laureates/2012/
4. Gati, C. *et al. IUCrJ* http://dx.doi.org/10.1107/S2052252513033939 (2014).
5. Emma, P. *et al. Nature Photon.* **4,** 641–647 (2010).
6. Chapman, H. N. *et al. Nature* **470,** 73–77 (2011).
7. Redecke, L. *et al. Science* **339,** 227–230 (2013).
8. Barty, A. *et al. Nature Photon.* **6,** 35–40 (2012).
9. Aquila, A. *et al. Opt. Exp.* **20,** 2706–2716 (2012).

MOLECULAR BIOLOGY

A second layer of information in RNA

Three studies have characterized the full complement of RNA folding in cells. They find large numbers of secondary structures in RNA, some of which may have functional consequences for the cell. SEE LETTERS P.696, P.701 & P.706

SILVIA B. V. RAMOS & ALAIN LAEDERACH

The RNA molecule is generally understood as a messenger of genetic information in the cell: it is transcribed from DNA and then translated into proteins[1]. Stretches of RNA that are complementary in sequence have a propensity to pair, forming elements of secondary structure, such as hairpin loops, within RNA molecules. But the prevalence of secondary structure in messenger RNAs, and its role in RNA regulation, is not fully understood. In this issue, three reports[2–4] describe analyses of all the mRNA molecules present in different populations of cells — transcriptome-wide analyses — using structure-probing techniques. These studies begin to reveal the extent of secondary structure in the transcriptomes of plants, humans and yeast.

The chemical structure of RNA is analogous to that of DNA. It is comprised of a sugar–phosphate backbone and four distinct nucleotide bases: adenine (A), cytosine (C), guanine (G) and uracil (U). As with DNA, these bases interact by forming hydrogen bonds, resulting in aptly named Watson–Crick pairs (G–C and A–U). However, unlike DNA, complementary bases from two RNA molecules do not pair up to form a double helix, a formation that in DNA prevents secondary structures from arising. Instead, the nucleotides of RNA are free to interact with one another within each molecule, resulting in folding of the RNA chain into secondary structures (Fig. 1).

The functional consequences of secondary structural elements in RNA depend on their molecular context. Some specific structural elements have well-known regulatory roles after gene transcription, but these are restricted to small subsets of mRNAs[5,6]. In some cases, such as in ribosomal RNA (part of the cellular

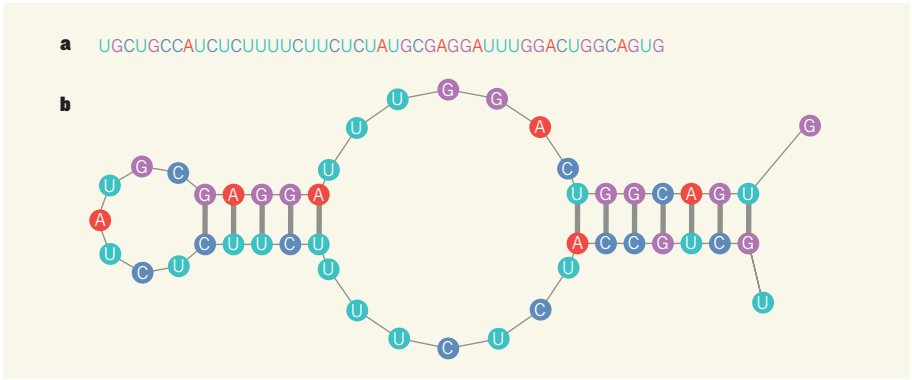

Figure 1 | Principles of RNA primary sequence and secondary structure. **a**, RNA is a single-stranded polymer, with nucleotide bases adenine (A), cytosine (C), guanine (G) and uracil (U). **b**, Unlike DNA, RNA molecules do not pair up to form helices. The bases of an individual molecule can therefore pair with one another (G–C and A–U), causing the RNA to fold into secondary structures. G bases can also pair with U bases, forming a G–U wobble pair. Three reports[2–4] find that such folding is commonplace in humans, plants and yeast. (Figure adapted from Fig. 3 of ref. 4.)

machinery that synthesizes proteins), secondary structural elements fold further into compact three-dimensional conformations that can catalyse reactions[7].

The three new studies, each analysing different cell populations, use a combination of well-established chemical and enzymatic structure-probing techniques for determining RNA secondary structure together with next-generation sequencing, a method that allows simultaneous sequencing of millions of stretches of nucleotides. Ding and colleagues[2] (page 696) examined seedlings from the plant *Arabidopsis thaliana*, Rouskin and co-workers[3] (page 701) investigated yeast, and both Rouskin *et al.* and Wan and colleagues[4] (page 706) report analyses of secondary structures in humans. All three papers report unprecedented coverage of the transcriptome[8]. In doing so, they demonstrate unequivocally that most mRNAs have a propensity to form secondary structures *in vitro*, in the absence of any other cellular components.

Each group reports that some of the RNA structures they observed *in vitro* were altered *in vivo*. In fact, Rouskin and colleagues found evidence in yeast that RNA structures in the cell are actively unfolded by proteins. Nonetheless, the papers show that structural patterns are evolutionarily conserved at several functional sites within RNA molecules. These results provide the first *in vivo* data to suggest that, if given the opportunity, RNA will fold. This is consistent with many previous *in vitro* studies[9] of RNA structure and folding. Because mRNA must be unfolded to successfully act as a messenger, the cell must therefore find ways to get around the folding problem.

In addition to their structural characterization of the human transcriptome, Wan and co-workers performed comparative structure probing in cell lines derived from a family trio (mother, father and child). In so doing, they were able to assess the structural consequences of natural human inter-generational genetic variation on the transcriptome, and discovered more than 1,900 single-nucleotide mutations that alter RNA structure. These experiments therefore yielded thousands of new putative 'ribosnitches'[7,10] — broadly defined as RNA sequences in which a specific single-nucleotide mutation alters structure[7]. Ribosnitches are analogous to bacterial riboswitches, which change structure on binding of a small molecule and regulate transcription or translation[11].

Because RNA structure has the potential to influence post-transcriptional processes in the cell, a subset of the putative ribosnitches could be functional. Indeed, mutations that disrupt certain RNA secondary structural elements can cause human disease[10]. Although the structural changes identified in Wan and colleagues' work are not by themselves indicators of malfunction — the three individuals studied are presumably healthy — the newly identified putative ribosnitches have the potential to help to identify mechanisms by which structural changes can give rise to disease, an exciting step forward.

The application of next-generation sequencing to the transcriptome has previously revealed the complexity of post-transcriptional regulatory networks[5]. The structural dimension of this complexity is now accessible with the publication of these three papers. Although the three studies reveal similar general structural features of transcripts, there are key differences in the specific features found by each approach. Such discrepancies may come from differences in experimental design, which can cause changes to the inherently dynamic structure of RNA. In this case, each study used different protocols for RNA extraction, library preparation and, in particular, determining levels of background noise. These experimental details must be taken into account when comparing structures discovered using the different approaches.

The trio of reports provides our first insight into the secondary structure of an entire transcriptome in eukaryotes — the class of organisms comprising plants, animals and fungi. However, a full characterization of transcriptome structure will require a concerted community effort, with an emphasis on standardization to allow quantitative comparisons of these data sets. Only then will it be possible to fully integrate these findings to determine the structural elements that are consequential in the transcriptome[12]. ■

Silvia B. V. Ramos *and* **Alain Laederach** *are in the Obstetrics and Gynecology Department and the Biology Department, University of North Carolina, Chapel Hill, North Carolina 27599-3280, USA.*
e-mails: alain@unc.edu; silvia_ramos@med.unc.edu

1. Crick, F. *Nature* **227,** 561–563 (1970).
2. Ding, Y. *et al. Nature* **505,** 696–700 (2014).
3. Rouskin, S., Zubradt, M., Washietl, S., Kellis, M. & Weissmann, J. S. *Nature* **505,** 701–705 (2014).
4. Wan, Y. *et al. Nature* **505,** 706–709 (2014).
5. Woodson, S. A. *Curr. Opin. Chem. Biol.* **12,** 667–673 (2008).
6. Dominski, Z. & Marzluff, W. F. *Gene* **396,** 373–390 (2007).
7. Halvorsen, M., Martin, J. S., Broadaway, S. & Laederach, A. *PLoS Genet.* **6,** e1001074 (2010).
8. Tijerina, P., Mohr, S. & Russell, R. *Nature Protocols* **2,** 2608–2623 (2007).
9. Shcherbakova, I., Mitra, S., Laederach, A. & Brenowitz, M. *Curr. Opin. Chem. Biol.* **12,** 655–666 (2008).
10. Martin, J. S. *et al. RNA* **18,** 77–87 (2012).
11. Tucker, B. J. & Breaker, R. R. *Curr. Opin. Struct. Biol.* **15,** 342–348 (2005).
12. Ulitsky, I. & Bartel, D. P. *Cell* **154,** 26–46 (2013).

CELL BIOLOGY

Potency unchained

Differentiated cells have been reprogrammed to an embryonic-like state using a physical stimulus. This treatment generates a new cell population that contributes to both the embryo and the placenta. SEE ARTICLE P.641 & LETTER P.676

AUSTIN SMITH

Cell specialization in mammals is essential for diverse functions, such as muscle contraction and nerve conduction. These specializations become fixed during development, and conversion between differentiated cell types seems to be extremely rare. However, in this issue, two studies by Obokata *et al.*[1,2] show that cells isolated from newborn mice lose their identity on exposure to mildly acidic conditions. Remarkably, instead of triggering cell death or tumour growth, as might be expected, a new cell state emerges that exhibits an unprecedented potential for differentiation into every possible cell type.

Studies on tissue regeneration in amphibians, reptiles and birds indicate that differentiated cells have some ability to dedifferentiate or to switch identity. Mammalian cells are more resistant, but fate conversion is observed in certain cancers. It was only with the cloning of Dolly the sheep[3], in which nuclear material from the mammary cell of an adult sheep was transferred into an enucleated egg cell to produce a cloned animal, that the capacity for complete reprogramming of the mammalian genome was confirmed. However, cloning does not convert whole cells.

Whole cells can be induced to switch identity by genetic manipulation. The introduction of certain transcription factors can in specific contexts rewire gene circuitry, leading to changes in cell specialization[4]. In 2006, the cell-identity and cloning research paths were unified through the discovery of a phenomenon known as induced pluripotency[5]: when mouse fibroblast cells were treated with a quartet of embryonic regulatory factors, a small percentage adopted the molecular and functional attributes of embryonic stem cells. The resulting induced pluripotent stem cells (iPSCs) had the dual abilities to self-renew indefinitely and to differentiate into all somatic cell types (Fig. 1a). Subsequently, iPSCs have