A look back: message in a test tube

People have been working with RNA for the better part of a century now. However, the earliest RNA preparation techniques were often inefficient and unreliable—involving boiling, extreme alkaline conditions or chemical denaturants—and thus often impeded progress toward better understanding of the molecule itself. One important challenge involved keeping RNA intact in the presence of ribonuclease; as early as 1920, scientists were aware that biological tissues often contain high concentrations of this robust and efficient enzyme[1], and the successful analysis of RNA depends greatly on the extent to which one can effectively protect it from degradation. Today, of course, a variety of single-solution systems are available for rapid RNA preparation, solutions that are typically based on the integration of two different classical nucleic acid preparation methodologies.

One of these methods, the now-venerable phenol-chloroform extraction, is actually the culmination of decades of working with the two reagents independently. Chloroform first came onto the scene for nucleic acid work in the 1930s, achieving 'fame' in the hands of M.G. Sevag, who observed that chloroform and protein seem to enter into an interaction in solution that excludes nucleic acids. By adding a small fraction of isoamyl alcohol to prevent foaming during the mixing process, Sevag developed a simple method for phase-dependent separation of nucleic acids from crude cell extracts[2]—and the resulting chloroform–isoamyl alcohol mixture became known as 'Sevag' as a result. Phenol came into use years later, when it was demonstrated how effectively phenol solutions dissolve organic tissue, while also rendering RNases inactive; the resulting solution also separates into phases, from which RNA can be readily separated from both DNA and proteins[3]. Klaus Scherrer and James Darnell further refined this approach with their paper describing the simple 'hot phenol' extraction of RNA from HeLa cells[4], which would remain a favored approach for years to come.

Meanwhile, in the 1950s, two research groups began working with an alternative compound, guanidinium hydrochloride, which proved an extremely powerful denaturant for proteins while leaving nucleic acids largely intact. The first group, Volkin and Carter[5], used the approach to purify calf spleen RNA, and successfully obtained reasonably pure RNA that nonetheless had a surprisingly low molecular weight. The second group, Grinnan and Mosher[6], used a similar approach on calf and rat liver, obtaining much higher molecular weights that correlated with earlier estimates suggesting a far larger polymer. It wasn't until later that it became clear that the first group had used low concentrations of guanidinium (2 M) that left some ribonucleases active, whereas the second group had used the now-standard 4 M concentration, keeping the RNA intact. Though this method became popular and proved highly effective in many cases, there were still some tissues—for instance, the pancreas, which has especially high levels of RNase activity—for which standard guanidinium preparation proved inadequate. To address this, John Chirgwin and colleagues developed an optimized approach in which RNA was isolated in 4 M guanidinium combined with the reducing agent 2-mercaptoethanol, and this was followed by cesium chloride gradient purification in an ultracentrifuge[7]. Chirgwin's group demonstrated successful purification of intact and biologically active RNA even from pancreatic samples, and his method became the method of choice for RNA preparation.

Of course, even this approach placed certain technical demands on its users. Polish scientist Piotr Chomczynski had used Chirgwin's method while at the US National Institutes of Health, but found it difficult to perform upon his return to Poland in 1982. As he would later recall drily, "At that time, the government in Poland was too distracted by other matters to support research activity and buy ultracentrifuges"[8]. He set about developing a simpler and more cost-effective approach to RNA purification, and it was here that the phenol- and guanidinium-based approaches both came into their own. Chomczynski built on a technique described previously by James Feramisco[9], and produced a lysis and purification solution incorporating phenol, chloroform and guanidinium hydrochloride into a single mixture that enabled easy purification of non-degraded RNA from animal tissues in a matter of hours[10]. Chomczynski quickly parlayed the published method into commercial success—launching a company for the production of RNA purification reagents—as well as scientific fame, as the author of what is now the most widely cited paper in recorded history, with nearly 55,000 citations on record as of 2005. Most importantly, the resulting method proved a tremendous boon to subsequent RNA research, allowing researchers to finally focus on experimentation instead of preparation.

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