Activities of the La protein required to maintain SRP RNA integrity

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Aim of project:
- Investigate the effect of ribosomal mutations, in proteins and rRNA, on 2A-driven translational recoding.

Background & Aim:
- The La protein plays key roles in cellular RNA metabolism. A major activity of the protein is binding to 3' terminal U-tracts on RNA, a feature of RNA polymerase III (pol III) transcripts; tRNAs, RNA components of RNAse P, the U6 small nuclear ribonucleoprotein, and 5S rRNA amongst others. La orchestrates maturation of these RNAs, many of which have multi-step processing pathways.
- The signal recognition particle is a ribonucleoprotein required for protein targeting that contains a pol III RNA as a key component.
- In this project the aim was to examine mutations affecting that La protein's RNA binding surfaces for effects that they have on the SRP RNA, a pol III transcript.

The La protein and its RNA binding surfaces:

1) The beta sheet (seen as yellow in figure 1) is a known RNA recognition motif (RRM) of the La protein.
2) The cleft observed in the La protein is responsible for the binding of 3' terminal U-tracts present on RNA transcripts.
3) Previous work has demonstrated that in human La the loop (coloured orange on the diagram below) has a key role in La protein function, likely through interacting with RNA via its positively charged lysine residues.

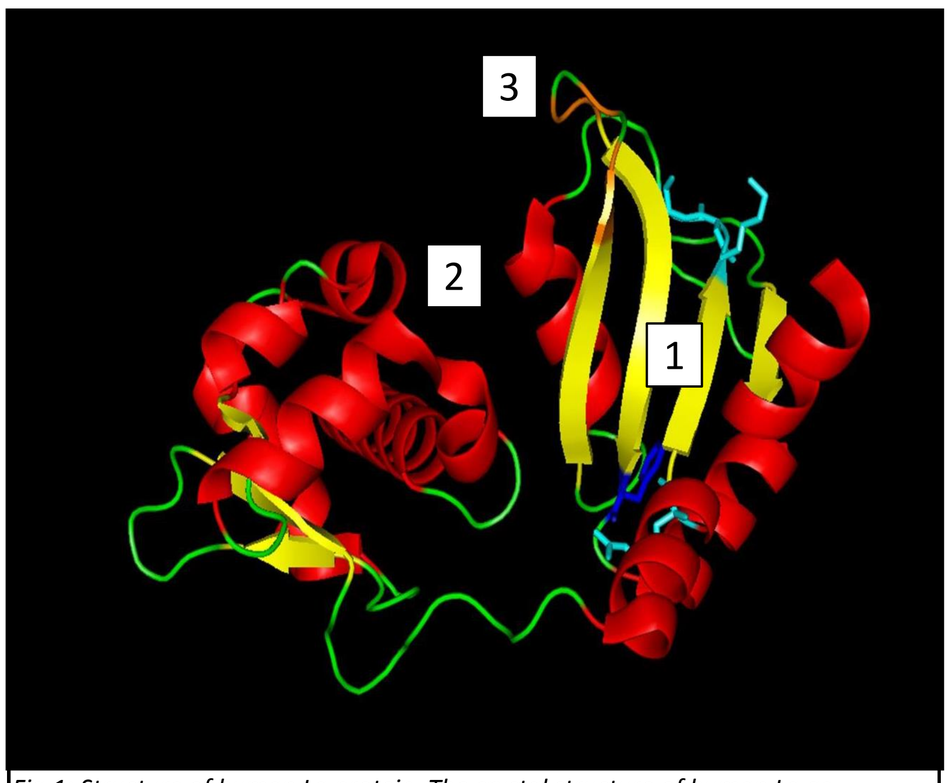


Fig.1: Structure of human La protein. The crystal structure of human La as determined by Kotik-Kogan et al. (2008) (NDB ID: 2VON). Structure was visualised and manipulated using PyMol molecular graphics.

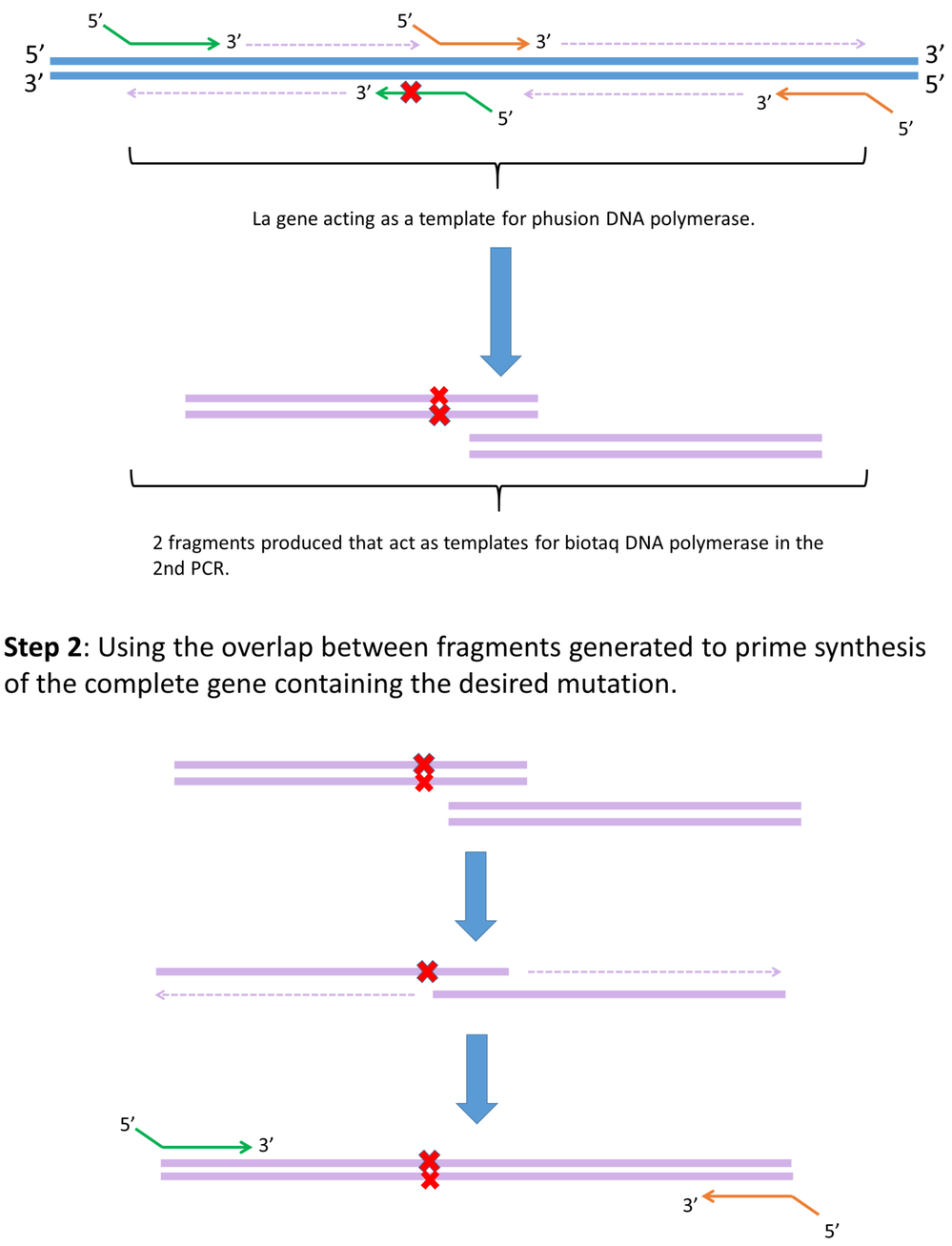
Using mutants to examine RNA binding sites of the La protein:
- In order to investigate the binding surfaces of the La protein we aimed to express versions of the La protein containing mutations in suspected or known RNA binding sites and observe the effects of these mutations on SRP RNA.
- Several plasmids containing La protein mutations were already available in the laboratory.
- For the purpose of this investigation the mutations of the La protein needed to be expressed in high and low copy plasmids. Some however were only present in either high or low plasmids and therefore new constructs were generated using cloning techniques.
- Additionally the L175A mutant was lacking altogether and had to be generated using PCR mutagenesis, the steps of which are outlined in figure 2.

Results:
- The cloned PCR product was sent for sequencing and proved to contain the L175A mutation.
- A collection of mutants were transformed into yeast. The Northern blot carried out verified that SRP RNA was expressed in the transformed cells (Figure 3). All except the F51A La mutant contained SRP RNA.
- Tried to look at U4 snRNA but unfortunately the blot did not work, possibly due to failure to label the probe.

Further work:
- Look more closely at SRP RNA and its processing when La mutants are present within cells.
- Repeat experiment and look at other cellular RNA.

2-Step Mutagenesis by PCR:

Step 1: Producing 2 over-lapping fragments of the La gene with one containing the desired mutation.



Step 2: Using the overlap between fragments generated to prime synthesis of the complete gene containing the desired mutation.

= amplification of whole gene containing mutation during 2nd PCR.

Fig.2: The process of mutagenesis by PCR.

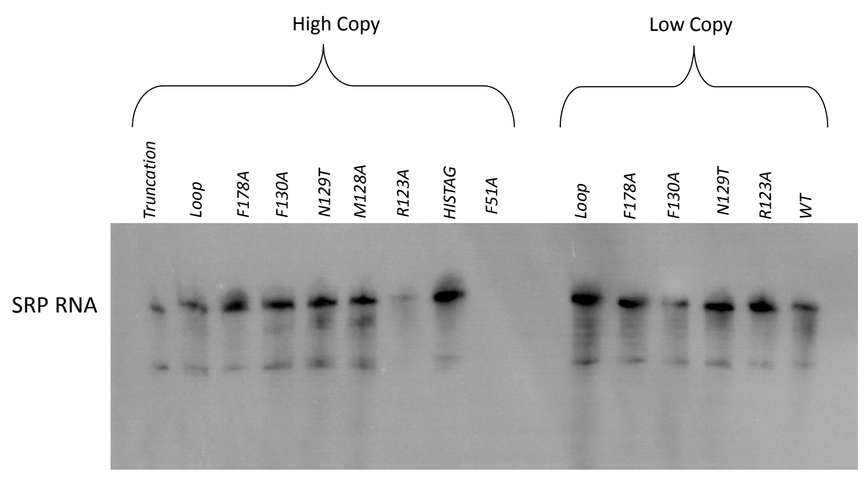


Fig.3: Northern blot of SRP RNA in cells with mutated versions of the La protein.

References:

Kotik-Kogan O, Valentine ER, Sanfelice D, Conte MR, Curry S. (2008) Structural analysis reveals conformational plasticity in the recognition of RNA 3' ends by the human La protein. Structure, 16:852-62.