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# The SHAPE of an IRES

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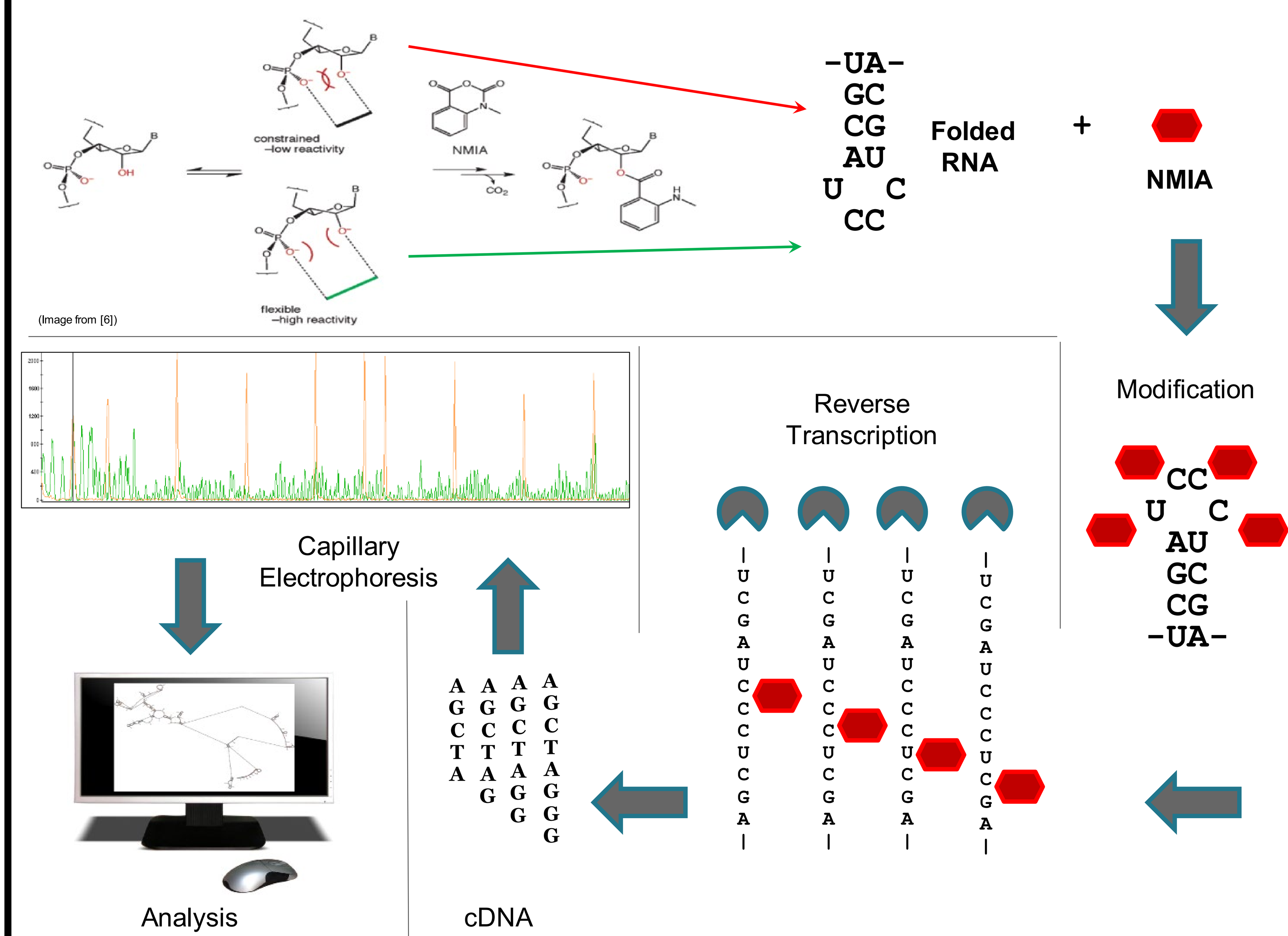
## Abstract

Internal ribosome entry sites (IRESs) are cis acting sequences located in the 5' UTR of some mRNAs that allow cap-independent translation initiation. IRESs are characterized by the formation of complex secondary structural features. An IRES has been proposed in the *Drosophila gurken* mRNA that allows production of its critical protein to continue under nutrient deprivation conditions, when cap-dependent initiation is inhibited. The mechanism and secondary structure of eukaryotic IRESs however remain poorly understood We use SHAPE chemistry to map the secondary structural elements of the *gurken* IRES and are working toward refining the structure to publication quality. To help elucidate the mechanism and the function of the *gurken* IRES secondary structures we have compared them to better understood viral IRESs. So far the secondary structure of the *gurken* IRES most closely resembles that found in type-2 viral IRESs.

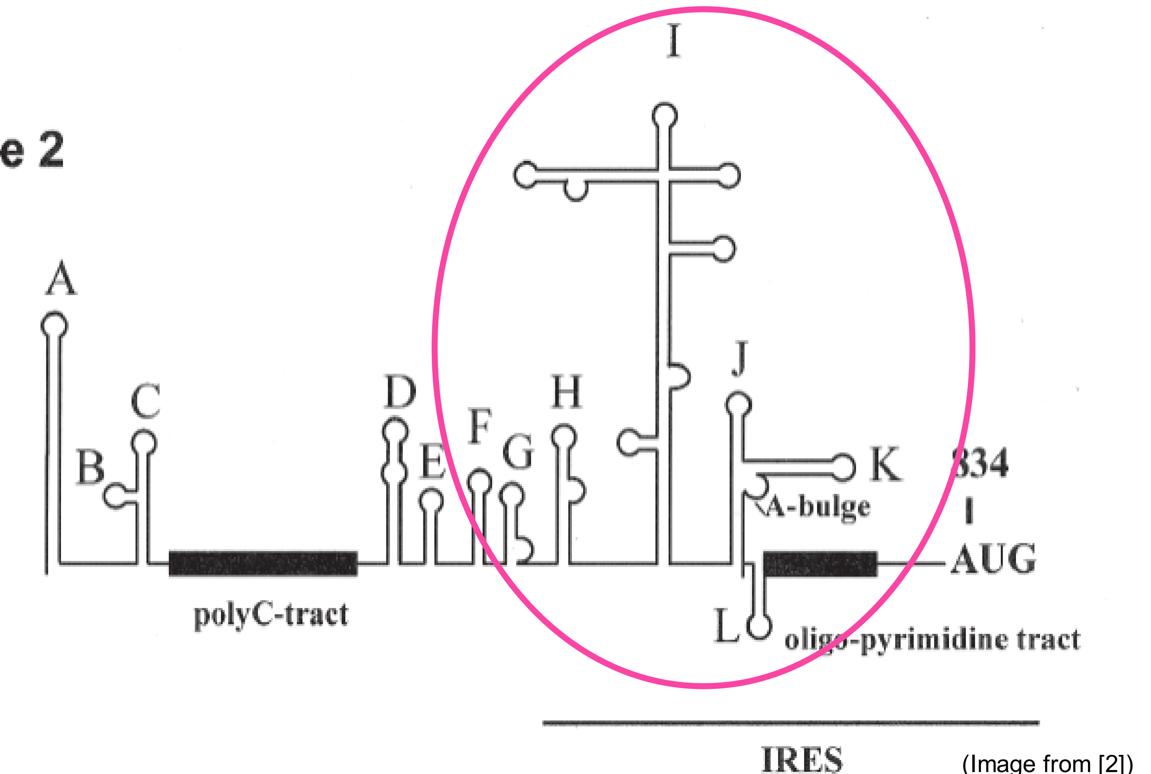
## Selective 2'-Hydroxyl Acylation Analyzed by Primer Extension




## Internal Ribosomal Entry Sites

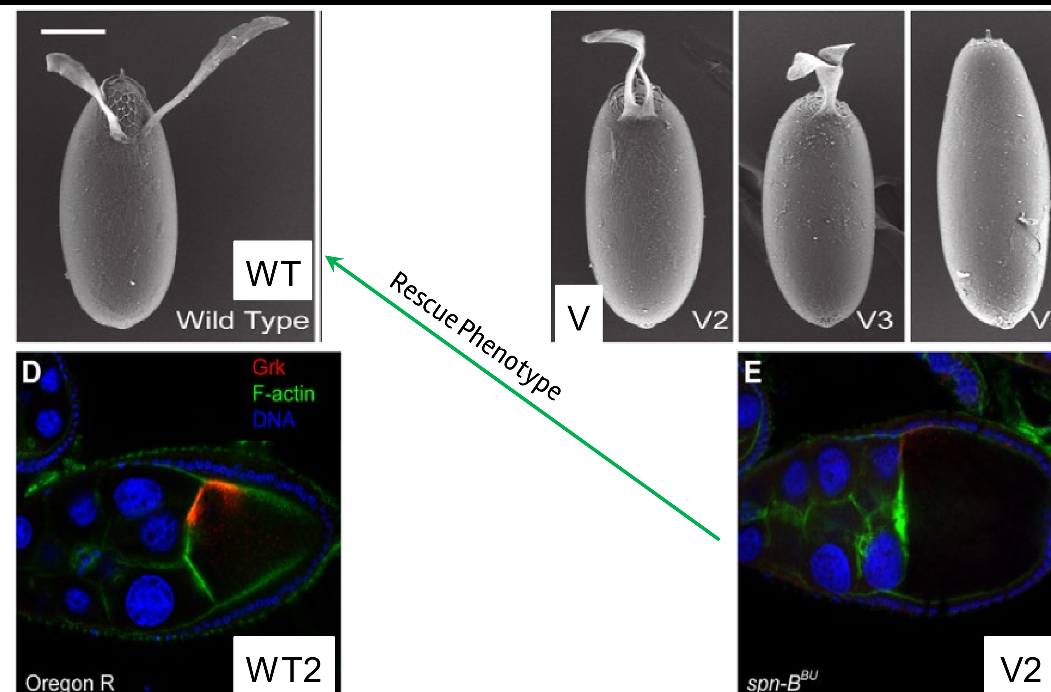
Internal ribosome entry sites or IRESs were first discovered in poliovirus RNAs, which lack a 5' cap and are unable to use canonical cap dependent translation mechanisms, but are still able to initiate translation[1]. IRESs can have wildly differing sequences and secondary structures, however are all cis-acting sequences located in the 5' UTR of mRNAs that actively recruit ribosomal subunits with few or none of the initiation factors required for cap-dependent translation[2,3]. Current research and understanding of IRES structures implicates them in two important functional roles. IRES structures are the means by which viruses hijack cellular translation machinery, and provide an alternative translation mechanism for some crucial eukaryotic mRNAs when normal 5' cap-dependent translation is inhibited. Eukaryotic IRESs are typically found in mRNAs that encode proteins that are critical for the organism's survival or reproductive functions. Viral IRESs, discovered first, have been well characterized and are categorized into several types that share secondary structure elements. A categorical system for eukaryotic IRESs does not yet exist.

**Schematic of a Type 2 viral IRES.** Type 2 viral IRESs are found in the genomes of aphthoviruses and cardioviruses, as well as hepatitis A and C which reside in a subclass of type 2 IRES[2]. The pink circled region is the most highly conserved among type 2 IRESs with the oligopyrimidine tract and A-bulge involved in binding IRES trans-acting factors. This is the viral IRES category most similar to our current structure.

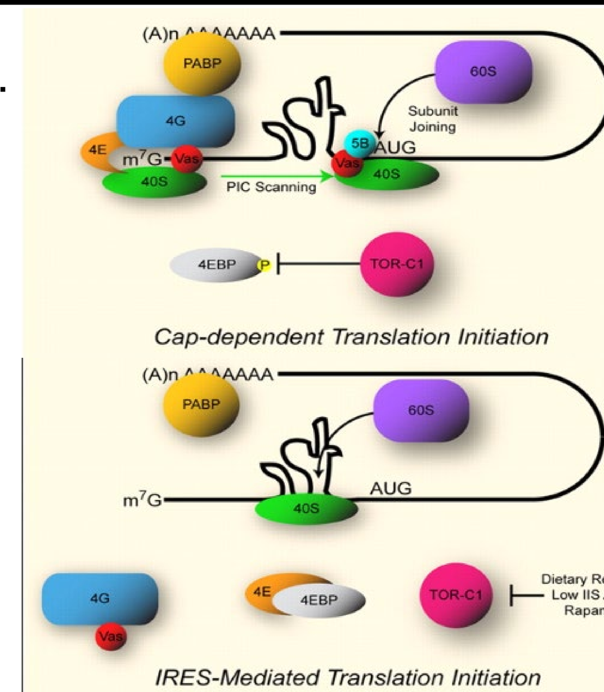



## Gurken

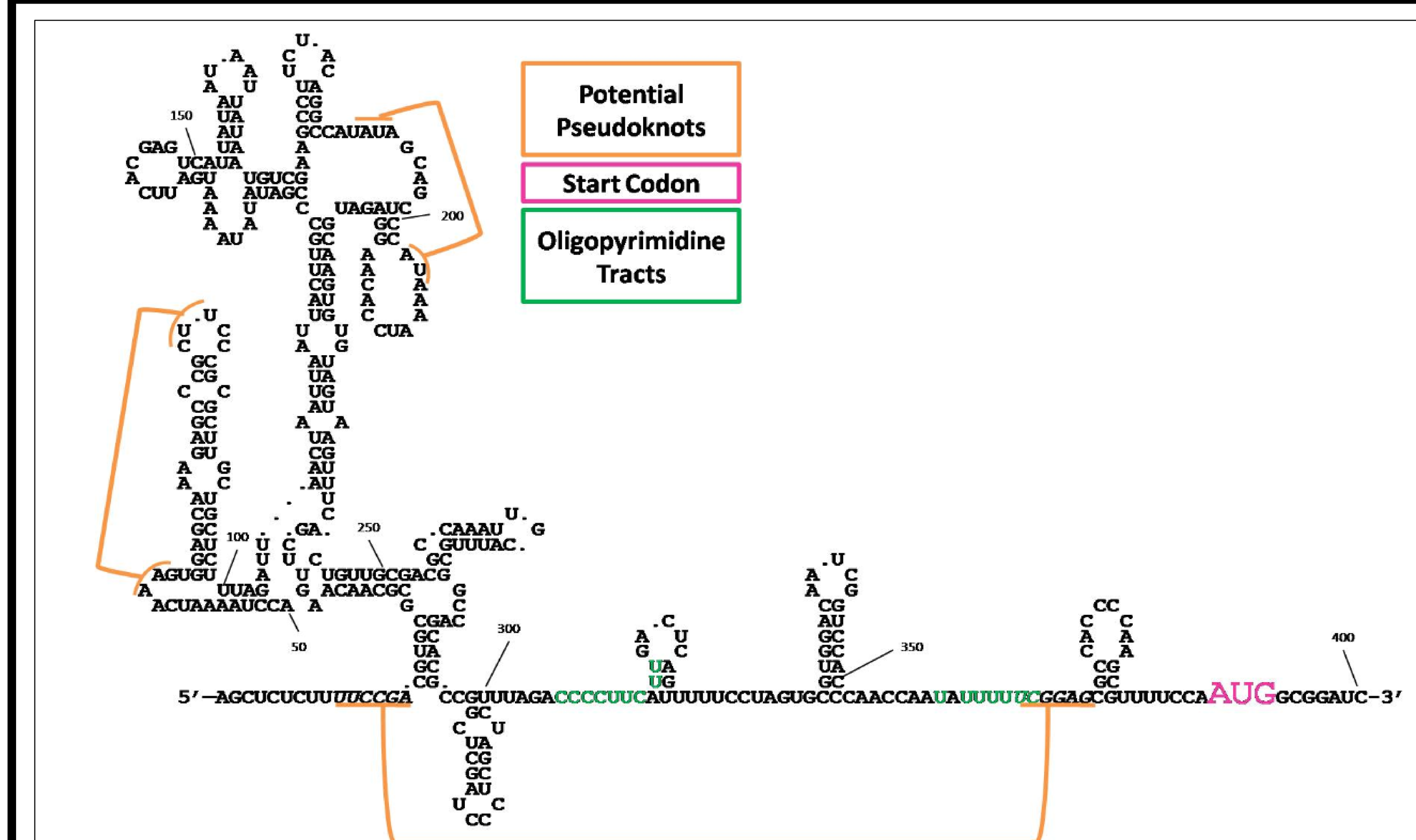
Gurken is an epidermal growth factor receptor (EGFR) ligand that plays a pivotal role in the establishment of dorsal/ventral polarity in developing oocytes during *Drosophila* oogenesis. During the mid stages of oogenesis in wild type *Drosophila* the *grk* mRNA becomes localized and translated in the future dorsal-anterior portion of the oocyte, functioning as a selective cellular signal to establish polarity and guide further oocyte development[4]. Several *gurken* mutations have been characterized that cause loss of function or loss of Gurken localization resulting in ventralized or dorsalized eggs respectively. One mutation, spn-B[BU], causes loss of function and ventralized egg formation[5]. Interestingly the phenotype can be rescued with treatments that block normal 5'-cap dependant translation, suggesting an alternative translation method.




**Wild type *gurken* and the effects of mutations.** (**WT**) Wild type dorsal appendage formation. (**WT2**) Confocal microscope image showing dorsal-anterior Gurken localization. (**V**) Progressive ventralization of eggs in spn-B[BU] *gurken* mutants, beginning with V2 and ending with completely ventral V4. (**V2**) Confocal image showing loss of Gurken. (Images from [5])




**Modes of *gurken* mRNA translation initiation.** Shown at the top is normal cap-dependant translation that relies on the m[7]G cap and a host of trans-acting initiation factors to recruit the 40S ribosomal subunit. At the bottom, dietary restrictions or Rapamycin treatment block cap-dependant translation, however the 40S is recruited directly by the cis-acting IRES structure. (Images from [5])




## Current Structure

The image on the left is our current structure of the *gurken* mRNA 5'-UTR IRES derived from multiple SHAPE data sets. This structure was obtained from applying SHAPE constraints corresponding to local nucleotide flexibility to a thermodynamically optimized base pairing algorithm[8]. The complex series of stem loops and potential pseudoknots near the 5' end of our structure are reminiscent of the highly conserved regions of Type 2 viral IRESs. Also similar to Type-2 viral IRESs, our structure has multiple oligopyrimidine tracts within 30-80 basepairs upstream of the start codon, shown in green. In Type-2 viral IRESs these oligopyrimidine tracts are the sites of IRES trans-acting factor binding, which help recruit and position the 40S ribosomal subunit directly on the start codon, so translation can initiate without scanning for the start codon. In other types of viral IRESs the oligopyrimidine tract is located 180+ nucleotides upstream of the start codon and the 40S ribosomal subunit must scan for the start codon before forming a translation competent 80S[2].

The most well characterized Type-2 IRES to date, from Hepatitis C, also has several regions of complementarity to the 18S rRNA upstream of the AUG start codon that has been shown through cryo EM studies of HCV-80S complexes. This complementarity guides the IRES start codon into the mRNA binding cleft through base pairing[3]. Recently we have found multiple regions of complementarity near the start codon of our structure to the *Drosophila* 18S rRNA, raising the possibility of a similar mechanism here.

## Future Directions

Future research will be focused on further refinement of the wild type *gurken* IRES structure, establishing IRES structure-function relationships by analyzing the secondary structure of mutant *gurken* 5' untranslated regions, and probing the effects of folding time on IRES secondary structure. Our colleagues at SUNY Fredonia have generated multiple *Drosophila* strains that have shown loss of IRES activity through in vitro functional studies. Using SHAPE chemistry the secondary structure of *gurken* IRESs that have lost the ability to initiate translation can be mapped, allowing us to correlate important secondary structures with IRES function.

## References

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