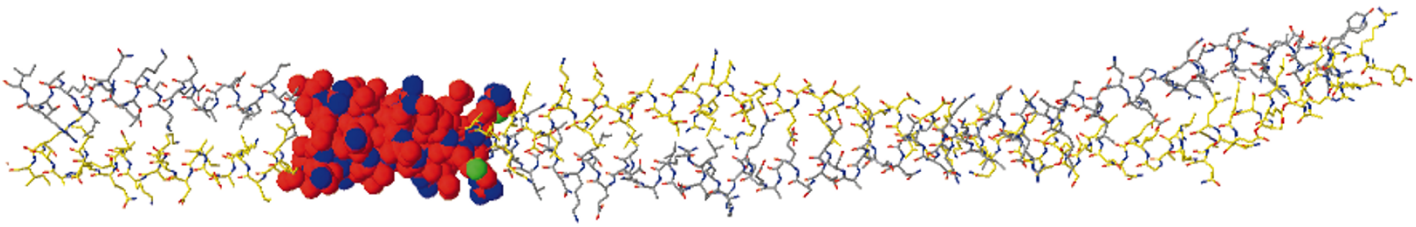
this induction of subgenomic RNA synthesis occurs even if RNA-2 does not encode a functional protein. Thus, it was reasoned that the RNA-2 molecule itself may play a role in activating transcription of the subgenomic RNA.

To determine which section of RNA-2 is necessary for this transcriptional activation, the researchers replaced the capsid protein gene on RNA-1 with the green fluorescent protein coding region (to allow easy visual assays of infected tissue) and utilized a heterologous viral RNA expression system in which RNA-2 could be analyzed by deletion mutagenesis. Using this strategy, they narrowed down the activation region to 34 nucleotides of RNA-2 that were predicted to form a stem-loop. Intriguingly, the eight nucleotide loop region was complementary to a region in RNA-1 just upstream of the transcription start site of the subgenomic RNA, suggesting that RNA-1 interacts with RNA-2 through base pairing of their respective eight nucleotide segments (see the top image for a model of the base pairing between RNAs). Mutations in one RNA region or the other destroy activation, but compensatory changes in both RNAs restore activation. The lower images clearly illustrate the results of infection experiments with various combinations of constructs containing wild type RNA-1 (R1sGFP), mutant RNA-1 (R1sGFP-M), wild type RNA-2 stem loop (pHST2-SL2) and mutant RNA-2 stem loop (pHST2-SL2-M); the altered nucleotides of the mutants are indicated in red. It is apparent that only the two wild type RNAs and the two complementary mutant RNAs can be paired to induce expression of the green fluorescent protein reporter, which indicates induction of the subgenomic RNA, strongly supporting the RNA–RNA interaction hypothesis. Despite obvious remaining questions (Do the paired RNAs form a specific structure? Are proteins involved? What is the exact mechanism of regulation?), it is clear that this type of activation makes sense with regard to viral infection. It ensures that sufficient quantities of RNA-1 and RNA-2 are present before the capsid protein is expressed — and before virion assembly begins. **TS**

OK, and the α-helix

After having focussed on the β-sheet in the Editorial (see pages 749–750), it would be difficult — nearly impossible, in fact — to further discuss some of the latest results in biomolecular structural biology presented at the recent American Crystallographic Association Annual Meeting without mentioning the α-helix, very much the 'first among equals' of protein secondary structure. Perhaps the most apposite example was provided by P. Burkhard (University of Basel, Switzerland) who reported on the structure determination of the 190 Å long α-helical, two-stranded, right-handed coiled-coil rod domain (Ir) from cortexillin I, an actin-bundling protein from *Dictyostelium discoideum* (shown below). This is the longest structure of a coiled coil reported to date, soundly beating the 39-residue long cFos-cJun bZIP leucine zipper.



Cortexillin I is comprised of a globular head domain and the rod, or oligomerization domain, the later of which consists of 18 heptad repeats. It is common knowledge that fragments of coiled coil domains very often do not associate into coiled coils, indicating that the heptad repeats are not sufficient to drive assembly of the quaternary conformation. What, then, was the secret of the success of the Ir structure determination? The rod domain includes a 13-residue 'trigger site' (space filling format above) that has been shown to be necessary for coiled coil assembly and, indeed, has been characterized as an autonomous folding unit. Biochemical experiments have suggested that electrostatic interactions are a critical component of the trigger site's structure (Steinmetz, M.O. *et. al. EMBO J.* **17**, 1883–1891; 1998). These observations are further supported by the structure, which shows that all 16 intramolecular salt bridges seen in the Ir structure are formed at the trigger site. A consensus sequence for the trigger site has been identified in other coiled coils and the Ir trigger sequence has been shown to function in the context of the GCN4 coiled coil (Krammerer, R.A. *et al.*, *Proc.Natl. Acad. Sci. USA* **in the press**), suggesting that this is a general feature of coiled coil assembly. **GR**