3. Goal setting

The "σ^S promoter selectivity paradox" has puzzled researchers for many years. How can RNAP (E) containing an alternative sigma factor, σ^s, bind optimally to promoter sequences in-vitro that are nearly identical to those recognised by the housekeeping RNAP, $E\sigma^{70}$ (Gaal et al., 2001), and nevertheless recognise an extensive and distinct set of genes in-vivo? The solving of this conundrum acquired a higher significance after it was realised that target overlap by sigma factors is a common feature of the transcriptional logic in bacteria. Already before the beginning of this thesis, some light had been shed upon features that made promoters σ^s-selective in *E. coli* (mostly due the work of my colleague, Gisela Becker; (Becker, 2003) but also from research done by other groups, summarised in Hengge-Aronis, 2002; see also Fig. 15). Nevertheless several important issues remained open or completely unexploited, and constituted the basis of my research in the past few years:

1. The presence of a distal UP-element sub-site in *csiD*p was previously found to be a stimulating feature for $E\sigma^s$–dependent transcription, whereas its completion to a full UP-element site enabled $E\sigma^{70}$ to utilise the promoter equally well (Germer et al., 2001). One of the first questions that arose was whether UP-element full or half-sites had a general impact in promoter selectivity. If yes, then how was this selectivity mediated, considering that UP-elements are contacted by αCTD and not the sigma factor. Do the two holoenzymes follow different ways for promoter recognition? And then which part of the sigma factor is responsible for such a differential behaviour?
2. One of the early beliefs considering σ^s-dependent promoters is that they lacked an apparent –35 element, and that $E\sigma^s$ did not need such a DNA determinant for promoter recognition (Espinosa-Urgel et al., 1996; Lee and Gralla, 2001). However, since i) all amino acids enabling σ^{70} to recognise the –35 element are conserved in σ^S, ii) $E\sigma^s$ bound stronger to a promoter with a perfect –35 hexamer in-vitro (Gaal et al., 2001), and iii) the deletion of a –35 element did not increase the $E\sigma^s$–selectivity of a synthetic promoter (Becker, 2003), it appeared that this concept should be revisited.
3. Class II type of activation usually requires an interaction between the sigma factor in RNAP and the adjacently bound activator protein (whereas in the bacterial class I transcriptional activation mechanism the activator contacts the alpha subunit). Could the unique outer face of σ^s, exposed to class II activators, selectively cooperate with

certain trans-acting factors? And then how would $E\sigma^{70}$ behave with the same transcriptional activators?

4. A prerequisite for $E\sigma^{s}$-derived transcription in stationary phase or upon stress encounter is that σ^{s} has to first successfully out-compete σ^{70} from the limiting amounts of core RNAP that the cell sustains. Are there any factors that directly aid σ^{s} in this competition? What is the role of Crl in this matter?