Although for the most part this chapter will be concerned with sequences that are transcribed in amphibian oocytes, studies in this field have progressed in such a way that transcribed sequences cannot be considered in isolation from sequences that are not transcribed. Neither can lampbrush chromosomes be considered in isolation, for work on other chromosomes has frequently supplied evidence that is essential for the proper appraisal of the primary topic of this chapter. Much of the information at present available has come from the use of in situ hybridization, the singularly valuable autoradiographic technique devised by Gall and Pardue (1969) and by H. A. John et al. (1969). Mention has been made of one outcome of this technique in the section of Chap. 3 dealing with nucleolus organizers, but here I will discuss wider aspects.

The first successful application of in situ hybridization to lampbrush chromosomes was made by Barsacchi and Gall (1972). They hybridized \(^3\)H-labelled RNA copied in vitro, after the removal of rDNA, from total \(N.\ viridescens\) DNA (cRNA) to denatured interphase nuclei, mitotic and lampbrush chromosomes of this newt. They obtained heavy labelling of heterochromatic lumps (chromocentres) in interphase nuclei, of regions adjacent to the centromeres of mitotic chromosomes, similarly of the axial regions without loops that include the centromeres of the lampbrush chromosomes, the regions of chromosome axes to which the spheres are attached, and some telomeric regions. They also noticed, in preparations from nearly mature oocytes that had been stimulated by gonadotropin, that the loops which in these circumstances become prominent at the sphere loci are also labelled. Because the RNA probe used in these first experiments was heterogeneous, the most heavily labelled regions of the chromosomes would necessarily be those containing the most highly repeated sequences complementary to the most highly repeated sequences present in the probe.

In situ hybridization with better characterized probes was soon under way, attention being first directed at target sequences in urodele genomes that are known, on biochemical evidence, to be highly reiterated. One such family of sequences is 5 S rDNA. Barsacchi Pilone et al. (1974) hybridized \(^3\)H-labelled 5 S rRNA prepared from \(Xenopus\) tissue culture cells to denatured interphase nuclei, mitotic and lampbrush chromosomes of \(T.\ marmoratus\). In mitotic chromosomes a single locus is labelled, intercalary in the long arm of chromosome X, and likewise a single locus in the main axis of lampbrush chromosome X, close to a landmark loop with dense matrix. In this paper Barsacchi Pilone et al. (1974) state that the 5 S genes are similarly confined to a single locus on the axis of chromosome
X of *T.c. carnifex*. The 5 S genes of *T. vulgaris meridionalis* are likewise concentrated at a single locus in the long arm of chromosome XI, and in situ hybridization to the lampbrush chromosomes of this species confirmed that they lie in one or a few chromomeres near the middle of this chromosome (Barsacchi-Pilone et al. 1977). Using iodinated 5 S rRNA on denatured mitotic and male meiotic chromosomes León (1976) found a single locus for 5 S rDNA sequences in *Taricha granulosa*, and two loci in *Batrachoseps wrighti*, in both species the loci lying close to centromeres.

Most of the 5 S genes in the karyotype of *N. viridescens* also lie close to centromeres; Hutchison and Pardue (1975) hybridized 3H-cRNA transcribed in vitro from 5 S rDNA of *Xenopus* to denatured mitotic chromosomes of *N. viridescens*. They found sites of hybridization adjacent to the centromeres of four pairs of subtelocentric chromosomes, and one other site in the middle of the long arm of a pair of one of the two smallest chromosomes. Pukkila (1975) confirmed these findings, and extended the investigation to the lampbrush chromosomes. Using 125I-labelled 5 S rRNA isolated from *N. viridescens* as a probe, and hybridizing to denatured preparations, she found labelling confined to the centromeric regions of four lampbrush chromosomes (I, II, VI, and VII) and, though less consistently, to an intercalary site on the long arm of chromosome X. The seven other centromeric regions were unlabelled.

Pukkila (1975) went a step further by hybridizing 125I-labelled 5 S rDNA prepared from *Xenopus* erythrocytes to non-denatured lampbrush preparations of *N. viridescens*. The intention here was to hybridize the DNA probe to nascent RNA, and loops near the centromeres of chromosomes I, II, VI, and VII were labelled. Control preparations digested with RNase before hybridization were unlabelled, whereas in preparations digested with DNase before hybridization, labelled loops were still observed. This seemingly convincing demonstration that 5 S RNA is transcribed on loops large enough to be seen by light microscopy raised problems that were recognized by Pukkila. The labelled loops ranged in length from 15 to 200 μm. However the 5 S rDNA coding sequences are so short (120 bp) that they can accommodate only one or at the most two polymerases at any instant, and the 5 S genes are separated from each other by spacer DNA (Kay et al. 1981, Kay and Gall 1981). So unless transcription were to proceed uninterrupted through many 5 S plus spacer sequences, the product would not be expected to form a lateral loop with enough matrix to be visible by light microscopy. Working with Miller-spreads Scheer (1982) has found clusters of highly repetitive, very short TUs, of the order 10,000 per cluster, arranged in tandem in *P. waltlii* (see Chap. 4). Each unit is covered by two polymerases only, the spacer intervals are likewise short, and Scheer considers it likely that these TUs represent 5 S rDNA. Though uninterrupted transcription through 5 S plus spacer sequence might account for Pukkila's labelled loops (see later), her findings were not confirmed in a study by Kay and Gall (unpublished).

Schultz et al. (1981) tackled this problem from a different angle. They developed an incubation medium (see Chap. 2) which supports RNA synthesis, extending over several hours, by isolated oocyte nuclei of *N. viridescens*. 3H-labelled ribonucleoside triphosphates were included in the incubation media, and in some experiments α-amanitin was also included, at 0.5 μg ml⁻¹ or 200 μg ml⁻¹. Xeno-